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Maternal effects on reproduction and development in the mud snail, *Ilyanassa obsoleta*

A thesis submitted in partial fulfillment of the requirement

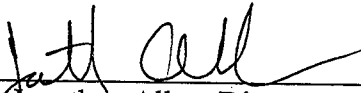
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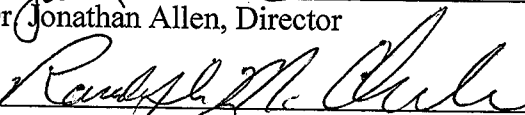
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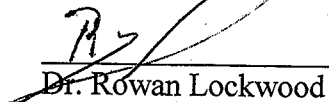
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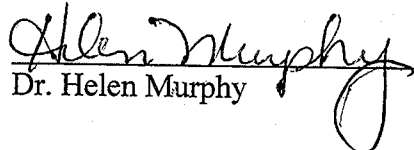
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**Maternal effects on reproduction and development in the mud snail, *Ilyanassa*
*obsoleta***

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April 13, 2012

Table of Contents

Abstract.....	4
Introduction.....	6
Methods.....	12
Summer 2010	
<i>Shell Size vs. Mass.....</i>	12
<i>Effect of Snail Size and Predator Presence on Egg Capsule Deposition.....</i>	12
<i>Egg Capsule Morphometrics.....</i>	14
<i>Snail Sex Determination.....</i>	14
<i>Effects of Snail Length and Sex on Intertidal Distribution.....</i>	14
Summer 2011	
<i>Effect of Snail Size and Predator Presence on Egg Capsule Deposition</i>	15
<i>Time to and Size at Hatching.....</i>	17
<i>Egg Capsule Morphometrics.....</i>	17
<i>Effect of Capsule Morphology on Predation.....</i>	17
<i>Statistical Analysis.....</i>	18
Results.....	19
Summer 2010	
<i>Shell Size vs. Mass.....</i>	19
<i>Egg Capsule Deposition in the Absence of C. Maenas.....</i>	19
<i>Egg Capsule Morphology in the Absence of C. Maenas.....</i>	20
<i>Egg Capsule Deposition in the Presence of C. Maenas.....</i>	21
<i>Egg Capsule Morphology in the Presence of C. maenas.....</i>	22
<i>Effects of Snail Length and Sex on Intertidal Distribution.....</i>	22

Summer 2011	
<i>Egg Capsule Number, Number of Eggs per Capsule, and Egg Size</i>	23
<i>Time to and Size at Hatching</i>	24
<i>Egg Capsule Morphometrics</i>	25
<i>Predation Trials</i>	26
Discussion	28
<i>Egg Capsule Number, Number of Eggs per Capsule, and Egg Size</i>	28
<i>Effects of Snail Length and Sex on Intertidal Distribution</i>	30
<i>Time to and Size at Hatching</i>	31
<i>Capsule Morphometrics: Height and Width</i>	33
<i>Capsule Morphometrics: Spine Length Plasticity and Predation Trials</i>	33
<i>Response of Small Snails to Urchins</i>	34
Conclusion	38
Acknowledgements	39
Figures	40
Tables	61
References	66

ABSTRACT

A maternal effect occurs when the phenotype of an organism is influenced by the phenotype of its mother. When the maternal environment is an accurate predictor of the offspring's environment, maternal effects can play an important role in enhancing offspring fitness. Maternal investment (e.g. egg size), which is often a function of maternal size, is one mechanism for the transmission of maternal effects. I used the mud snail, *Ilyanassa obsoleta*, as a model system for examining the effects of maternal size on offspring phenotypes. Females deposit egg capsules on blades of eel grass, and exhibit high variability in the number of egg capsules laid, the number of eggs per capsule, and the morphology of their egg capsules. During early ontogeny, encapsulated embryos suffer high levels of predation. I conducted two studies to test for the presence of inducible maternal effects in response to predation. First, I conducted a pilot study analyzing how maternal size affects egg capsule deposition in small (shell length = 14.5 - 18.0 mm) medium (18.1 - 21.0 mm) and large (21.1 - 26.0 mm) snails, both with and without the presence of a predator (the green crab, *Carcinus maenas*). In this experiment I measured egg size, the number of eggs per capsule, the number of egg capsules laid, and several egg capsule morphometrics. I then, in a more complete study, investigated how egg capsule deposition and embryonic development are influenced by maternal size in the presence of predatory green crabs by exposing small (15 – 19 mm) and large (21 – 25 mm) adult mud snails to *C. maenas* cue and measuring egg size, egg number, egg capsule number, and egg capsule morphometrics. Additionally, I measured larvae at hatching to test for effects of predator cues on intracapsular development. Across both studies, I found that large snails lay more egg capsules and eggs per capsule, and that large snails lay significantly larger eggs than small snails. Larval size at hatching increased significantly in the presence of green crabs, and there is a trend suggesting

that these larvae hatch sooner. Egg capsules were longer and wider in large snails, and, in the presence of a predator, were wider and possessed significantly longer defensive spines. In a test of predator preference, capsules with short spines were preferentially preyed upon by hermit crabs, suggesting that spines may be an adaptive deterrent to predation on egg capsules.

Altogether, these results suggest that maternal effects in *I. obsoleta* can be size- and context-dependent, play an important role in defending embryos from predators during early development, and may persist post-hatching.

INTRODUCTION

Phenotypic plasticity is defined as the ability of an organism of a single genotype to produce multiple phenotypes under different environmental conditions (Pigliucci, 2001). Plasticity is a widespread mechanism for generating responses to environmental heterogeneity, often through the production of variable morphologies and life histories within a single species (West-Eberhard, 1989). Producing phenotypic changes in a particular trait can be costly, potentially trading off with other traits such as fecundity and growth (Stearns, 1989; Williams, 2001). However, plastic responses frequently serve an adaptive function, and allow organisms to mount appropriate responses to a broad range of environmental challenges, including abiotic factors (Hamdoun et al., 2003), competition (Pfennig et al., 2006; Allen et al., 2008), and predation (Trussell and Smith, 2000; Laurila et al., 2002).

Predator-induced plasticity is often triggered in response to predator kairomones (i.e. interspecific chemical cues that benefit the receiver; Ruther et al., 2002), and can lead to the formation of morphological defenses that deter predation (Harvell, 1990; Trussell, 1996). For example, in the presence of an insect predator, adults of the cladoceran, *Daphnia cucullata*, have been shown to develop an elongated helmet and tail-spine (Tollrian, 1990). These long spines mitigate predation by significantly lowering the rate at which a number of gape-limited predators can prey upon *D. cucullata* (LaForsch and Tollrian, 2004). Similarly, in response to cues from a predatory dragonfly, larval gray treefrogs (*Hyla chrysoscelis*) undergo changes in body shape that allow them to swim faster, potentially enabling them to escape predation (McCollum and Leimberger, 1997). However, predator-induced plasticity is not limited to morphological defenses, and can be expressed as behavioral responses at different stages of development. For example, eggs of the red-eyed treefrog, *Agalychnis callidryas*, are laid on vegetation

overhanging temporary ponds. When egg masses are attacked by cat-eyed snakes, *Leptodeira septentrionalis*, developing larvae hatch prematurely, dropping into the water below and escaping from the immediate threat of predation (Warkentin, 1995). In adults of species such as the Siberian jay (*Perisoreus infaustus*), nest visitation is decreased at times of day when predator presence is high, potentially minimizing the likelihood that these predators detect the nest and prey on the hatchlings inside (Eggers et al., 2006). These examples demonstrate that predator-induced plasticity can be affected by a number of proximate mechanisms at many stages of life history.

In early ontogeny, plastic responses to predators are frequently mediated by maternal effects (Bernardo, 1996). Maternal effects refer to the causal role of maternal phenotype in shaping offspring phenotype (Wolf and Wade, 2009). When maternal environment is an accurate predictor of offspring environment, maternal effects can promote rapid, adaptive responses by mothers that increase offspring survival. For example, in addition to inducing adult *D. cucullata* to form elongated helmet and tail-spines, insect predators also signal these adults to induce the same defenses in their offspring (Agrawal et al., 1999). In this way, maternal effects can be transgenerational in nature, allowing mothers to adaptively utilize information about their reproductive environment to modulate phenotypic variation in offspring (Fox and Mousseau, 1996; Mousseau and Fox, 1998; Agrawal, 2000). For example, maternal pea aphids, *Acyrtosiphon pisum*, increase the production of winged offspring when exposed to predators and parasitoids, which allows them to disperse away from the high predation pressure (Sloggett and Weisser, 2002). Besides predation, maternal effects can be mediated by a number of other factors, including competition, maternal diet, and maternal size (Stratton, 1989; Bellinger et al., 2004; Marshall & Keough, 2004).

Maternal size influences maternal effects when size directly influences offspring development through changes in maternal investment (e.g. egg size; Marshall & Keough, 2004). Maternal size effects are strongest in species lacking parental care, where maternal size is frequently a reliable predictor of offspring size (e.g. Sakai and Harada, 2001; Marshall et al., 2010). For example, in the colonial bryozoan, *Bugula neritina*, a reduction in the size of the maternal colony leads to a reduction in the size of the larvae produced, suggesting a positive correlation between maternal and larval size (Marshall & Keough, 2004a). In *B. neritina* and other marine invertebrate species, larger offspring size can influence a number of life history variables, including time to and size at settlement, post-settlement survival, growth rate, and even time to first reproduction (Marshall and Keough, 2003; Emlet and Sadro, 2006). This is true of the intertidal snail, *Nucella ostrina*, for which larger size at hatching results in increased larval growth rate, shorter time to maturity, and higher post-settlement survivorship (Moran and Emlet, 2001). These and other studies demonstrate the ways in which body size can modulate any of several ecologically relevant and plastic characters, but also the utility of snail species as model organisms for studying phenotypic plasticity (Trussell, 1996; DeWitt, 1998; Padilla, 1998).

The mud snail, *Ilyanassa obsoleta*, is an intertidal gastropod inhabiting mudflats from the Gulf of St. Lawrence to northern Mexico (Gosner, 1978). Across this range, *I. obsoleta* can be found in high abundance, with up to 1,000 adults m⁻² (Fell et al., 1982; Cranford, 1988; Schwab and Allen, unpublished data). Reproductive adults of this species show significant size variation between the largest (~26 mm) and smallest (~14 mm) snails (Scheltema, 1962; Schwab and Allen, unpublished data). During the reproductive season, *I. obsoleta* deposit approximately 100 egg capsules, with each containing between 30 and 300 eggs (Brenchley, 1982; Ritschof et al., 2002; Allen and Schwab, unpublished observations). Embryos develop inside capsules with no

parental care for approximately two weeks, at which point larvae secrete a hatching enzyme that allows them to exit the egg capsule through the capsule plug (Pechenik, 1975; Sullivan and Mangel, 1984). Hatchlings then disperse as obligately feeding veliger larvae for two to eight weeks before settling on the benthos (Scheltema, 1961; Scheltema, 1962; Brenchley, 1982).

As an encapsulated larva, *I. obsoleta* is preyed upon across its range by blue crabs (*Callinectes sapidus*), green crabs (*Carcinus maenas*), hermit crabs (*Pagurus longicarpus*), and periwinkles (*Littorina littorea*); as an adult, snails are primarily preyed upon by crab species including blue and green crabs (Tagatz, 1968; Brenchley, 1982). Given this diversity of predators, it is not surprising that mud snails express a range of plastic responses that mitigate the threat of predation during their life cycle. For example, when exposed to blue crabs, adult mud snails decrease the size of their shell aperture relative to shell length. This response appears to limit the degree to which blue crabs can insert their claws into the shell, likely increasing handling time and decreasing feeding success (Santoni and Allen, in preparation).

Given their immobility and high nutrient content, mud snail egg capsules are also frequent targets for predation (Brenchley, 1982). To mitigate predation, maternal snails and their offspring might employ a number of plastic responses during hatching and early larval development (Oyazarzun and Strathmann, 2011). For example, some gastropod species are able to decrease egg size, promoting faster development, earlier hatching, and dispersal from the maternal environment (Perron, 1981). Alternatively, larvae may be able to behaviorally respond to predation. For example, in the nudibranch, *Phestilla sibogaein*, early hatching by embryos can be initiated due to disruption and scattering of these embryos due to messy foraging by a crab predator (Strathmann et al., 2010). This early hatching likely allows larvae to mitigate future predation by escaping into the comparatively safer water column (Vaughn and Allen, 2010).

However, earlier hatching is not the only way that marine organisms can reduce predation. In the gastropod *Nucella emarginata*, egg capsules with thickened walls are more resistant to predation by co-occurring isopod species (Rawlings, 1994). In addition, nudibranch species are well known for depositing toxic, defensive metabolites within their egg masses that deter potential predators (Pawlik et al., 1988; Johnson and Willows, 1999). Further, *I. obsoleta* has been demonstrated to form an elongated spine at the apex of egg capsules in response to *P. longicarpus* cue (Allen and Santoni, 2009, unpublished data).

Taken together, these data suggest that mud snails may employ any of several plastic, inducible defenses in order to protect larvae during early ontogeny. However, the role that maternal effects play in the formation of these defenses is unknown, as are the conditions under which they occur. Maternal effects are a rarely studied phenomenon in intertidal organisms, despite their well-established importance in ecology and evolution (Bernardo, 1996; Mousseau and Fox, 1998). Further, few studies have shown how mothers and their larvae respond to the threat of predation during intracapsular development, or whether these responses can affect larval development and life histories across generations.

As an abundant intertidal snail that develops under significant and consistent predation pressure, *I. obsoleta* is an excellent model system for studying context-dependent and transgenerational maternal effects. Further, because reproductive females vary significantly in size, maternal size is likely to play a role in determining maternal investment. Here, I set out to test how maternal effects on reproduction and larval development are mediated by both maternal size and predator presence, using the predatory green crab, *C. maenas*, as a focal predator. *C. maenas* induces behavioral responses in both bivalves (Freeman and Byers, 2006) and gastropods (Trussell, 2000), and is known to prey upon mud snail egg capsules (Brenchley,

1982), making it an excellent predator for testing hypotheses about predation on *I. obsoleta*.

Specifically, I hypothesized that (1) larger snails will produce more eggs and egg capsules; (2) larger snails will lay larger egg capsules; (3) the presence of *C. maenas* will induce snails of all sizes to invest less in reproduction; (4) the presence of *C. maenas* will induce the formation of an elongated capsule spine as a defensive mechanism; (5) larvae will hatch sooner and at a smaller size in the presence of *C. maenas*.

METHODS

Summer 2010

Shell Size vs. Mass

Adult mud snails (*Ilyanassa obsoleta*) were hand collected from a mudflat adjacent to the Bowdoin College Coastal Studies Center (CSC) on Orr's Island (43° 79' N, 69° 95' W), ME in June of 2010. To determine whether shell length was an appropriate proxy for soft tissue mass (maternal size), snails were haphazardly sampled across a large size range and their shell length was measured using digital calipers. The shell of each snail was then cracked using a C-clamp, and both soft tissue and shell were weighed using an analytical balance. Samples of shell and soft tissue were then placed into a drying oven at approximately 180°C until all water content was removed. Dry and wet soft tissue mass were then regressed against shell length using the power function to determine the strength of the relationship between shell length and maternal size.

Effect of Snail Size and Predator Presence on Egg Capsule Deposition

After confirming shell length as an appropriate proxy for maternal size, shell lengths of snails were measured to assess size variation above 14.5 mm (the size when *I. obsoleta* becomes reproductive; Scheltema, 1964) using digital calipers.

In June of 2010, 600 snails were divided equally by shell length into small (14.5 – 18.0 mm), medium (18.1 – 21.0 mm), and large (21.1 – 26.0 mm) size classes. The lower limit for these size classes was based on the size at which snails become reproductive, while the upper limit was based on the maximum size found in early surveys of mud snail size variation (Scheltema, 1964; Schwab and Allen, unpublished data). The 200 snails of each size class were further divided into replicate flow-through plastic containers (232mm L x 168mm W x 89mm

H), for a total of 30 containers housing 20 snails each. Each container was asymmetrically divided by a mesh barrier, with snails contained in the larger section (147.5mm L x 168mm W x 89mm H; Figure 1). The smaller section (70mm L x 168mm W x 89mm H) was left empty in this experiment. Snails were fed one blue mussel, *Mytilus edulis*, once every two weeks. Overall, the goal of this experiment was to assess the variation in reproductive output between size classes in this population, with snails only exposed to background levels of predator cue from the flow-through seawater. To collect pilot data on the ways in which predators influence reproductive output in mud snails, I conducted a second month-long pilot study, this one beginning in July, for which a new set of snails was exposed to a predator confined to the formerly empty section of the containers.

In July 2010, 600 snails were divided equally by shell length into small (14.5 – 18.0 mm), medium (18.1 – 21.0 mm), and large (21.1 – 26.0 mm) replicate plastic containers housing 20 snails each. Half of the replicates of each size class were exposed to a green crab predator (*Carcinus maenas* (46 – 56mm carapace width) collected from the rocky intertidal zone adjacent to the CSC. Green crabs were placed on the opposite side of the mesh container as previously described, allowing for the diffusion of crab kairomones while protecting snails from predation (Figure 1). Both mud snails and green crabs were fed one *M. edulis* every two weeks.

A removable mesh insert (insert size: 1.1mm L x 150mm W x 85mm H; square pore size: 1.16 mm radius) was placed along the mesh barrier, on which snails oviposit. To assess egg capsule deposition, the mesh insert was removed and egg capsule number was quantified daily to investigate the rate at which egg capsules were laid. Once larvae had hatched, each egg capsule was removed via forceps and counted as part of a final egg capsule tally for each container. To assess egg number per capsule, 10 freshly-laid egg capsules were removed from the mesh of

each replicate and examined under a dissecting microscope at 10x magnification. The top of each egg capsule was removed using a razor blade, and eggs were gently squeezed out of the capsule onto a glass slide using forceps and counted. Slides were then moved to a compound microscope where eggs were measured at 100x magnification.

Egg Capsule Morphometrics

To assess the size of the egg capsules, 10 capsules from each replicate were removed at approximately five days post-laying and measured for capsule height, capsule width, spine length, and the ratio of spine length to height using the ocular micrometer of a dissecting microscope at 10x magnification (Figure 2). By measuring the ratio of spine length to height, we hoped to investigate allometry in egg capsules, as well as whether changes in spine length might be correlated with other capsule morphometrics such as height.

Snail Sex Determination

Following data collection, and at the end of the snail reproductive period, snails were destructively sampled using a C-clamp. Snail sex is cryptic in *I. obsoleta*, and only direct observation of gonad color can sufficiently determine the sex of a snail. This procedure made it possible to determine the sex ratio in each replicate and obtain an accurate measure of the number of egg capsules laid per female. Snails were scored as male if their gonad was a dark-orange to red in color or as female if the gonad was a cream to light-orange color (Figure 3; Sternberg et al., 2008).

Effects of Snail Length and Sex on Intertidal Distribution

To assess spatial distribution and size-based demography of the mud snail population, three vertical transects were laid across the CSC mudflat (Figure 4). Using a 1 m² quadrat, the

number of snails along each transect was successively counted from the high to the low tide line. If a small number of snails were contained within a quadrat, all snails within that quadrat were collected and measured for shell length and destructive sampling for sex ratio determination. If a large number of snails were present in a quadrat, they were sub-sampled using a 0.25 m² quadrat placed haphazardly within the quadrat. All snails within the 0.25 m² quadrat were collected and measured for shell length and then destructively sampled for sex ratio determination.

Summer 2011

Using pilot data from 2010, I began a more complete and effective study in summer 2011 investigating how egg capsule deposition and embryonic development are influenced by maternal size in the presence of a predator.

Effect of Snail Size and Predator Presence on Egg Capsule Deposition

Adult mud snails were hand collected from a mudflat adjacent to the Bowdoin College CSC on Orr's Island, ME in June of 2011. 600 snails were divided equally by shell length into small (15 – 19 mm) and large (21 – 25 mm) size classes. The exclusion of a medium size class and the increased size range for large and small size classes was part of an effort to increase the number of replicates I could run with the two size classes that were most likely to differ in response variables. 300 snails of each size class were then sorted randomly into one of 15 replicate flow-through plastic containers, for a total of 30 containers housing 20 snails each. Each size class included five replicate containers of each treatment group, including a predator (green crab, *C. maenas*; 46 – 56mm carapace width), a metabolic control (the green urchin, *Strongylocentrotus droebachienses*; 25 – 34 g), and a control (Figure 1). The function of the metabolic control was to ensure that the response of mud snails to green crabs was not simply a

response to sharing water with a heterospecific, but rather a specific response to an ecologically relevant predator. We selected green urchins to function as the metabolic control because (1) they are generally herbivorous, and do not prey upon mud snails or their egg capsules, and (2) they do not share a habitat with mud snails. As such, we expected that mud snails would respond similarly to the presence of green urchins as they would to control treatments. Predators and metabolic controls were contained on the opposite side of the mesh divider as described for summer 2010; however, all experimental animals were fed one *M. edulis* twice per week for four weeks rather than once per week in 2010. This measure was taken to ensure all snails were consistently fed.

The mesh insert was removed and egg capsule number was quantified daily in order to investigate the total number of and rate at which egg capsules were laid. Egg capsules were removed via forceps following hatching and counted as part of a final egg capsule tally for each container. To assess egg number per capsule, 10 freshly-laid egg capsules were removed from the mesh of each replicate and examined under a dissecting microscope at 10x magnification. The top of each egg capsule was removed using a razor blade, and eggs were squeezed out of the capsule onto a glass slide using forceps. Slides were then moved to a compound microscope where eggs were counted at 100x and measured at 250x magnification.

Following data collection, snails were destructively sampled using a C-clamp to determine the sex ratio in each replicate and obtain an accurate measure of maternal investment per female (Figure 3). I then used the derived sex ratio to determine the number of egg capsules laid per female. By multiplying this number by the average number of eggs in each egg capsule, I was able to calculate the total reproductive output in terms of the number of eggs per female in each replicate.

Time to and Size at Hatching

To assess time to hatching, egg capsule deposition and larval hatching were tracked throughout the duration of the experiment. The time at which larvae hatched was determined by the difference between the time when 50% of capsules were laid and the time when 50% of capsules had hatched.

To assess size at hatching, five capsules from each replicate were isolated near hatching and placed into individual wells within a plastic well plate. Following hatching, larvae were immediately transferred to 1.7 ml microcentrifuge tubes and preserved in 70% ethanol and filtered seawater. Fixed larvae were then transported from the Bowdoin College CSC to the College of William and Mary in Virginia. The shell length of each veliger larva was measured using a Zeiss Achromat S Microscope[®] to assess size at hatching (Figure 5).

Egg Capsule Morphometrics

To assess the size of the egg capsules, 30 capsules per replicate were removed at approximately five days post-laying and measured for capsule height, capsule width, spine length, and the ratio of spine length to height using the ocular micrometer of a dissecting scope at 10x magnification (Figure 2).

Effect of Capsule Morphology on Predation

To test whether capsule spines deter predation, 10 small-spined (0 – 375 μm) and 10 large-spined (575 μm or greater) egg capsules were glued at random positions on a blade of eel grass using Krazy Glue[®] gel, which in previous studies was shown to cement the reproductive stages of marine invertebrates without deterring predators (Figure 6; JD Allen, personal observation). Each piece of eel grass was isolated in a plastic container housing either one hermit

crab (*Pagurus longicarpus*), one green crab, or one green urchin (*Strongylocentrotus droebachiensis*). Predators were allowed to feed on egg capsules until 50% were consumed or until 24-hours had elapsed since the beginning of the trial, at which point the individual predator was replaced if it had not begun feeding. The average height, width, and spine length of capsules both before and after predation were then compared to detect which variables significantly influenced predator foraging decisions.

Statistical Analysis

For the regression analysis plotting shell size against two measures of body mass, I utilized an adjusted R^2 value. I conducted two-way ANOVAs for the following dependent variables: egg capsule number per female, egg number per capsule, total number of eggs produced, egg size, capsule morphometrics, and larval time to and size at hatching. Where subsamples were available, ANOVAs were nested. In each of these ANOVAs, size (large, medium, or small) and treatment (green crab, urchin, control) and the interaction between size and treatment were modeled as fixed effects. When there was a significant treatment effect I conducted Bonferroni post-hoc tests to analyze differences among treatment levels. Prior to analysis with ANOVA, the distribution of all dependent variables was tested for normality using a Kolmogorov-Smirnov test. Additionally, I used a logistic regression to model predator preference for egg capsules using three variables: capsule height, width, spine length. Following regression analysis, I used a Hosmer and Lemeshow test to find the goodness of fit of the model. All statistical analyses were completed using SPSS statistical software, version 18.

RESULTS

Note: Where they overlap between studies, the statistical results of each measure are described in Table 1.

Summer 2010

Shell Size vs. Mass

I regressed wet body weight (Figure 7) and dry body weight (Figure 8) against shell length using a variety of functions including cubic, exponential, linear, logarithmic, logistic, polynomial, and power. Overall, I found that the power function best fit the data, with an adjusted R^2 of 0.612 for wet body weight and 0.627 for dry body weight. Both of these regressions were statistically significant ($p < 0.001$ for both measures). From this, I concluded that shell length was a reliable, though imperfect, predictor of maternal body mass.

*Egg Capsule Deposition in the Absence of *C. maenas**

In this experiment, all 10 large snail replicates produced egg capsules. However, only nine medium and seven small snail replicates produced egg capsules.

When accounting for the number of snails that were female in each plastic container (12.77 ± 0.86 females, Mean \pm SE; ascertained via destructive sampling and sex determination), I found that egg capsule number varied significantly with size class (ANOVA, $F_{2,23} = 5.518$, $p = 0.011$). However, this result was driven mostly by the difference in egg capsule number between large (79.4 ± 6.5 capsules) and small snails (30.7 ± 11.5 capsules; Bonferroni post hoc test, $p = 0.009$). Differences between large and medium (55.9 ± 4.7 capsules; $p = 0.297$) and medium and small snails were not significant ($p = 0.326$; Figure 9).

The number of eggs deposited per capsule varied significantly with snail size (Nested ANOVA, $F_{2,100} = 11.362$, $p < 0.001$). Medium snails laid more eggs per capsule (195.3 ± 7.4

eggs) than small snails (154.8 ± 4.3 eggs; Bonferroni post hoc test, $p < 0.001$). Additionally, large snails (178.9 ± 7.5 eggs) laid significantly more eggs per capsule than small ($p = 0.010$) but not medium ($p = 0.114$) snails (Figure 10). Additionally, there was a significant effect of container number when nested within snail size (Nested ANOVA, $F_{22,100} = 1.814$, $p = 0.025$).

Size classes significantly differed in their total reproductive output per female during the reproductive season (ANOVA, $F_{2,23} = 8.044$, $p = 0.002$). Large and medium snails produced significantly more eggs (14134.3 ± 1341.3 eggs and 11980.2 ± 714.0 eggs, respectively) than small snails (6753.8 ± 1750.1 eggs; Bonferroni post hoc test, $p = 0.002$ and $p = 0.034$, respectively); however, there was no difference in the number of eggs produced by large and medium snails ($p = 0.679$; Figure 11).

Egg Capsule Morphology in the Absence of C. maenas

Egg capsule height varied significantly with snail size (Nested ANOVA, $F_{2,223} = 19.666$, $p < 0.001$). Large and medium snails both laid capsules that were taller (1993.6 ± 32.7 μm and 2154.3 ± 22.7 μm , respectively) than those of small snails (1958.4 ± 53.7 μm ; Bonferroni post hoc test, $p < 0.001$ for both measures). However, capsule height did not differ between large and medium snails ($p = 1.000$; Figure 12). Further, there was no effect of container number when this variable was nested within maternal size ($F_{23,223} = 1.233$, $p = 0.219$).

Egg capsule width varied significantly with snail size (Nested ANOVA, $F_{2,223} = 26.120$, $p < 0.001$). Capsule width did not differ between large and medium snails (2131.7 ± 43.0 μm and 2064.9 ± 25.9 μm , respectively; Bonferroni post hoc test, $p = 0.226$). However, large capsules were significantly wider than small capsules (1829.2 ± 28.7 μm ; $p < 0.001$), as were medium capsules ($p < 0.001$; Figure 13). There was additionally an effect of container number when this variable was nested within maternal size ($F_{23,223} = 1.595$, $p = 0.046$).

Finally, spine length did not vary with snail size (Nested ANOVA, $F_{2,201} = 0.524$, $p = 0.113$; Figure 14), and there was no effect of container number when nested within maternal size ($F_{23,201} = 1.364$, $p = 0.132$). Additionally, neither the ratio of spine length (Nested ANOVA, $F_{2,201} = 0.136$, $p = 0.873$; Figure 15) to height nor the effect of container number ($F_{23,201} = 1.546$, $p = 0.059$) varied with maternal size.

Egg Capsule Deposition in the Presence of C. maenas

In this experiment, nine large, six medium, and zero small snail replicates deposited egg capsules.

The number of egg capsules laid per female (17.4 ± 0.8 females per container) varied significantly with snail size (Large Snails: 64.2 ± 4.3 capsules; Medium Snails: 44.9 ± 7.9 capsules), but not with crab presence or absence. Further, there was no significant interaction between snail size and crab presence (Table 2; Figure 16).

Egg number per capsule was not affected by either crab presence or snail size. Further, there was no interaction between the two, and no effect of container number when this variable was nested within maternal size nested within treatment (Table 2; Figure 17).

Large snails did not have a greater reproductive output than medium snails, though reproductive output did decrease when green crabs were present (Control: 12063.6 ± 697.1 eggs; Green Crab: 9140.9 ± 1597.2 eggs). However, there was no interaction between size and crab presence/absence (Table 2; Figure 18).

Egg Capsule Morphology in the Presence of C. maenas

Egg capsule height varied significantly with snail size, but not green crab presence. Further, there was no interaction between these two variables, and no effect of container number when this variable was nested within maternal size nested within treatment (Table 3; Figure 19).

Egg capsule width varied significantly with snail size (Large Snails: $2162.6 \pm 22.9 \mu\text{m}$; Medium Snails: $1985.4 \pm 24.8 \mu\text{m}$), though not with green crab presence or absence. Additionally, there was no interaction between these two variables, and no effect of container number when this variable was nested within maternal size nested within treatment (Table 3; Figure 20).

Both spine length and the ratio of spine length to height did not vary with either snail size or green crab presence/absence, and there was no interaction between those two variables and no effect of container number when this variable was nested within maternal size nested within treatment (Table 3; Figure 21; Figure 22).

Effects of Snail Length and Sex on Intertidal Distribution

In transects 1 and 3, snail density peaked at 1.15 meters below the high tide line, suggesting that snails in these regions are most dense at a higher point in the intertidal than snails in transect 2, which were densest at 1.3 meters below the high tide line. The mean snail density across all three transects was highest at 1.15 meters, as per transects 1 and 3 (Figure 23).

Both transects 1 and 3 showed a general increase in snail size along their range as the distance from the high tide line increases. Transect 2 showed a bimodal distribution, with the largest snails found at either extreme of its range. The overall mean shows a bimodal distribution, with the largest snails being found at less than 1 and 1.65 meters below the high tide line (Figure 24).

For all transects, males tended to become more frequent with decreasing tidal height. The number of data points above the mean line in Figure 25 suggests that males predominate in the population, which may relate to the fact that males are generally smaller than females (Figure 26; Schwab and Allen, unpublished observation).

Biomass was negatively correlated with shell length, and positively correlated with snail density and the proportion of males in the population (Figure 27). This suggests that males are small, dense, and comprise most of the biomass in the population, while the inverse appears to be true for females. Overall, the highest biomass appears to be found at the mid-point of each transect, and increases with distance from the rocky shoreline that green crabs inhabit (Figure 27).

Summer 2011

Egg Capsule Number, Number of Eggs per Capsule, and Egg Size

When accounting for the number of snails that were female in each plastic container (12.9 ± 0.8 females), I found that the number of egg capsules laid per female varied significantly with size. Large snails (152.8 ± 10.0 capsules) laid significantly more egg capsules than small snails (112.6 ± 17.0 capsules). There was no effect of treatment (Table 4). However, there was a significant interaction between snail size and treatment. This interaction was driven by the urchin treatment, where large snails once again laid more egg capsules when compared to the control, while the small snails laid less (Table 4; Figure 28).

There was a significant difference between large (184.5 ± 6.1 eggs) and small snails (144.7 ± 4.9 eggs) in the number of eggs deposited per capsule, but not by treatment (Table 4). However, there was a significant interaction between snail size and treatment, and a significant

effect of container number when this variable was nested within maternal size nested within treatment (Table 4; Figure 29).

Overall, large snails had a greater total reproductive output per female (28186.4 ± 2019.3 eggs) during the reproductive season than small snails (16016.2 ± 2316.6 eggs), though this number was not affected by treatment (Table 4). The interaction between snail size and predator presence was only slightly non-significant, but showed a similar pattern to that seen in both egg capsule results described above (Table 4; Figure 30).

Large snails produced larger eggs (158.8 ± 0.2 μm diameter) than small snails (157.7 ± 0.5 μm diameter), though this effect was only seen in the green crab and urchin treatments, where eggs were approximately 2% larger (Nested ANOVA, $F_{1,2619} = 64.917$, $p < 0.001$), and produced a significant effect of treatment (Nested ANOVA, $F_{1,2619} = 11.710$, $p < 0.001$; Figure 31). Additionally, there was a significant interaction of size and treatment (Nested ANOVA, $F_{261,2619} = 19.769$, $p < 0.001$), driven largely by the control treatment, and a significant effect of container number when this variable was nested within maternal size nested within treatment (Nested ANOVA, $F_{25,2619} = 23.082$, $p < 0.001$).

Time to and Size at Hatching

The time at which larvae hatched was neither affected by maternal size (ANOVA, $F_{1,26} = 0.010$, $p = 0.919$) nor treatment (ANOVA, $F_{2,25} = 1.359$, $p = 0.276$). However, although these results are statistically non-significant, they are suggestive of a trend towards earlier hatching in both the green crab and urchin treatments. Larvae from parents experiencing green crab (13.4 ± 1.0 days) and urchin (13.9 ± 1.4 days) cue hatched several days earlier than those not experiencing either (15.7 ± 0.8 days; Figure 32).

The size at which larvae hatched was significantly affected by both predator presence (Nested ANOVA, $F_{2,841} = 21.789$, $p < 0.001$) and maternal size (Nested ANOVA, $F_{1,841} = 13.234$, $p < 0.001$). In the presence of green crab cue, larvae hatched at a larger size than controls (Bonferroni post hoc test, $p < 0.001$), but not urchins ($p = 0.366$), although urchin larvae were also larger than controls ($p < 0.001$; Figure 33). Finally, I found a significant effect of container number when this variable was nested within maternal size nested within treatment (Nested ANOVA, $F_{23,841} = 3.857$, $p < 0.001$)

Egg Capsule Morphometrics

Large snails laid taller capsules than small snails, with large snail capsules (2241.8 ± 20.0 μm) being approximately 360 microns taller than small snail capsules (1882.1 ± 26.3 μm ; Nested ANOVA, $F_{1,860} = 513.642$, $p < 0.001$); however, capsule height was not affected by predator presence (Nested ANOVA, $F_{2,860} = 2.273$, $p = 0.104$; Figure 34). Yet, there was a significant interaction between snail size and predator presence (Nested ANOVA, $F_{2,860} = 11.958$, $p < 0.001$), driven once again by small snails in the urchin treatment, which appeared to increase capsule height relative to green crab and control treatments (Figure 34). Additionally, there was a significant effect of container number when this variable was nested within maternal size nested within treatment (Nested ANOVA, $F_{24,860} = 3.911$, $p < 0.001$).

Capsule width was significantly affected by snail size (Nested ANOVA, $F_{1,860} = 310.763$, $p < 0.001$), with large snails producing wider capsules than small snails (2046.3 ± 12.7 μm and 1797.4 ± 16.8 μm , respectively; Figure 35). Additionally, there was a significant effect of predator presence (Nested ANOVA, $F_{1,860} = 4.234$, $p = 0.015$), with snails exposed to crabs laying significantly wider capsules than in the urchin treatment (Bonferroni post-hoc test, $p = 0.029$). This helped to drive an interaction between snail size and treatment (Nested ANOVA,

$F_{1,860} = 7.010$, $p = 0.001$), where small snails exposed to urchins produced wider capsules than small snails in both the green crab and control treatments (Nested ANOVA, $F_{1,860} = 4.234$, $p = 0.015$; Figure 35). Additionally, there was a significant effect of container number when this variable was nested within maternal size nested within treatment (Nested ANOVA, $F_{24,860} = 1.665$, $p = 0.024$).

Interestingly, while there was no effect of snail size on the spine length of their capsules, there was a significant effect of predator presence (Table 5; Figure 36). Spines in the green crab treatment were significantly larger than those in the urchin (Bonferroni post hoc test, $p < 0.001$) and control ($p < 0.001$) treatments, while the latter two were not significantly different from one another ($p = 0.530$). When compared to control treatments, spine length increased by 25% (+107.78 μm) for small snails and 34% (+156.39 μm) for large snails. Additionally, snail size interacted with treatment group, where the presence of urchin cue resulted in small snails laying larger capsule spines than large snails exposed to urchin (Table 5; Figure 36). These results were mirrored when assessing the ratio between spine length and height; however, this variable was also affected by maternal size (Table 5; Figure 37).

Predation Trials

The discovery that spine length increases in the presence of green crabs verified observations from previous studies using hermit crabs (Santoni and Allen, unpublished data). While in Maine, I initially tested whether this induced response might serve an adaptive function by observing the feeding behavior of hermit crabs, green crabs, and green urchins on long-spined, free-floating capsules in the lab. In nearly all observed instances of predation by hermit and green crabs, egg capsules were sliced open near the capsule base (proximal end), with all eggs removed (Schwab and Allen, unpublished observations). Capsules were rarely entered near

the capsule plug (distal end), where elongated spines were located. There was no predation by green urchins.

Capsule predation trials from December 2011 suggest that hermit crabs selectively prey upon egg capsules on the basis of spine length. Green crabs did not show selectivity, and frequently ate all available capsules within a short period of time (Schwab and Allen, unpublished observations). Because of this, I only present data from hermit crab predation trials.

In December 2011, hermit crabs preferentially fed on short-spined capsules in seven of ten trials conducted (Figure 38). This suggests that spine length significantly influences hermit crab foraging decisions, with an increase of every 1 μm in spine length decreasing the likelihood of capsule predation by 0.2% (Logistic regression, $\text{Exp}(\beta) = 0.998$, $p = 0.011$; Table 6). Further, the foraging decisions that hermit crabs made were not driven by the position ($\text{Exp}(\beta) = 0.993$, $p = 0.800$), height ($\text{Exp}(\beta) = 0.999$, $p = 0.217$), or width ($\text{Exp}(\beta) = 1.000$, $p = 0.791$) of each capsule. A Hosmer and Lemeshow test of this logistic regression model was non-significant ($p = 0.738$), suggesting significant fit of the data. However, a small sample size rendered the ability of this model to predict predation at only 67%.

DISCUSSION

Egg Capsule Number, Number of Eggs per Capsule, and Egg Size

Large snails had a higher reproductive output than small snails in 2010, though they laid the same number of egg capsules as small snails in control and green crab treatments in 2011. It is unclear why this does not match results from 2010, and it is not immediately clear why large snails, whose energetic reserves are almost certainly greater, would not produce more egg capsules. Indeed, there is a general correlation between egg number and female body size in both gastropods and a number of other taxa (Barnes and Barnes, 1968; Honek, 1993; Ito, 1997). However, when assessing the total reproductive output and the number of eggs laid per capsule in 2010 and 2011, both years follow the same trend, with large snails producing significantly more gametes than small snails. This suggests that large snails invested more in eggs than egg capsules relative to small snails in 2011, which the data presented here confirm.

It should be noted that (1) egg capsule production was much higher in 2011 than 2010, and that (2) small snails significantly suppressed capsule laying in the presence of *Strongylocentrotus droebachiensis* in 2011. The increased egg capsule production in 2011 is likely due to differences in the amount of food that snails were offered, and potentially seasonality. In 2010, snails were fed one blue mussel every two weeks, while they were fed one blue mussel twice per week in 2011. Seasonality might explain the lack of capsule production by small snails in July 2010, as large and medium snails laid an equivalent number of egg capsules in both June and July experiments. Therefore, small snails may be investing all their reproductive energy into the early months of the breeding season, and potentially curtailing reproduction in July in order to invest in growth for future seasons. Although I collected data on small snail reproduction in 2011, these data are confined to the month of June. By July 2011, both large and

small experimental snails had finished laying egg capsules, as mud snail seasonal reproduction is synchronous and occurs when the titre of egg laying hormone is sufficiently high (Rittschof, 2002). Finally, the reason why small snails suppressed laying in 2011 may be related to the threat that green urchins pose to their productivity and survival (see below).

In 2011, large snails laid larger eggs than small snails, and these eggs were larger in control than in green crab and urchin treatments. This is not too surprising, as gastropods are known to frequently produce eggs of significantly variable sizes (Ito, 1997; Collin and Salazar, 2010). On average, there was a 2% increase in total egg volume between large and small snails. Although a small change, it is possible that this minor increase could be significant to the biology and early development of larval *I. obsoleta*. For example, if this 2% is allocated to yolk or other important developmental factors, larger eggs may develop into larger larvae. Indeed, although the difference is not a significant one, larvae of large snails were larger than the larvae of small snails in green crab and urchin treatments ($p = 0.078$; Figure 33). Further, the data presented here suggest a correlation between egg size and size at hatching, which is a common relationship amongst gastropods (Amio 1963; Ito, 1997). In addition, larger mothers increase reproductive output in all measures of fecundity (capsule number, egg number, egg and egg capsule sizes). These increases may simply be the byproduct of being a larger size during the reproductive season and having larger energetic reserves. Further, these results could reflect reproductive trade-offs in small snails between growth, capsule number, the size and number of eggs, and the size of capsules (e.g. Glazier, 1992).

It should be noted that many variables were significant for the nested effect of container number on maternal size and, in some cases, treatment group. These results suggest that the genetic and relevant phenotypic composition of individuals in separate containers were a

significant contributor to variation in the parameters measured. While this does not invalidate the results presented here, it is important to note this source of variation as one driver of the differences seen between replicates, and suggests that the haphazard sorting method utilized here may have been insufficient.

Effects of Snail Length and Sex on Intertidal Distribution

There was effectively no overlap in snail density between each transect at a particular tidal height, though there were similar patterns of density change along each transect (Figure 23). Snail density is initially very low, rises exponentially, and then drops as another, adjacent transect becomes dense in snails. Such a pattern forms a diagonal of snails at high-density across the intertidal, and may be the result of predation pressures. Parallel to the mudflat and transect lines is a hill composed of boulders, which is a refuge for green crabs at low tide. Transect 1 is nearest to these boulders, while transect 3 is farthest (Figure 4). In transect 1, snails were more densely distributed deeper into the intertidal than in transects 2 and 3. Because the densest patches of snails also contain the smallest snails, which are the most vulnerable to predation by crabs (Figure 24). It may be that crabs do not venture this low in the intertidal, or it may be that they spend less time foraging at lower tidal heights simply as a result of having to travel further to reach those hunting grounds. Interestingly, the distance from the green crab habitat increases from transects 1 to 3, and in tandem with small snail density, suggesting that crab foraging effort may decrease with distance from their home site. Altogether, because the Mussel Beach mudflat is relatively small, and because mud snails are highly mobile, these data suggest that the positioning of the small snails may be to avoid the areas where crabs forage most frequently (Schwab and Allen, unpublished observations).

Results from the analysis of body mass and sex ratio suggest that the smallest snails along the mudflat are males. Further, when taking snail density into account, this also suggests that males may outnumber females significantly in this population. It is presently unclear what mechanism has produced this interesting body size-density-sex ratio pattern. One possibility is that the biology of this species allows females to grow to larger sizes on average than males. This might explain the relationship between sex ratio and size in the population, since most females would be in the larger size classes and be difficult to detect amongst the smaller size classes. Additionally, females could grow at a faster rate than males and, thus, maintain an even sex ratio overall but still be generally larger. This may be possible, given that female mud snails appear to have enhanced foraging capabilities during the reproductive season (Curtis, 1979). Alternatively, mud snails may be sequential hermaphrodites, where males undergo a switch in gender with increasing size. This is not unheard of amongst gastropods, where hermaphroditism occurs in 3% of all prosobranch species, and it is interesting to note that males make up 77% of the population at 19 mm, before suddenly dropping to 41% of the population at 20 mm (Figure 26; Heller, 1993). These data suggest a potential size-dependent switch in sex from male to female that occurs around 20 mm. However, because snail sex is cryptic and requires the destruction of the shell (which is fatal to mud snails; Curtis and Hurd, 1983) it is unclear how this question can best be resolved.

Time to and Size at Hatching

The results presented here suggest that there is no significant difference in the time to hatching between snails exposed to green crabs, urchins, and controls. However, there is a trend for larvae to hatch sooner when exposed to predators, with larvae exposed to green crabs hatching 2.3 days sooner than controls. Developmentally, this is a significant result, given that

larvae tend to hatch after 14 days of development (Pechenik, 1975; Schwab and Allen, unpublished observations). Whether the reduced time to hatching is affected by maternal effects, larval hatching plasticity, or some mixture of the two is unclear, as larvae were continually exposed to green crab cue throughout their intracapsular development. Regardless, this result suggests that larvae may undergo early hatching as an adaptive response to the high threat of predation. Such a response would allow larvae to mitigate the threat of green crab predation by hatching and dispersing into the comparatively safe water column (Vaughn and Allen, 2010).

Larvae of large mothers hatched at a larger body size than those of small mothers, while larvae exposed to green crab cue hatched at a body length that was approximately 2.27% larger than controls. Therefore, despite hatching earlier and from smaller eggs, these larvae were also able to grow to a larger size, suggesting an increased rate of development in larvae exposed to predator cues. While there are potential benefits to being a larger size at hatching (e.g. a larger velum for increased feeding success, larger size at and earlier time to settlement and sexual maturity; Perron, 1981a; Marshall and Keough, 2003; Emlet and Sadro, 2006), it is unclear why control larvae do not also grow to this larger size. It may be that the large velum associated with larger size allows larvae to escape from the maternal environment quickly. As a tradeoff, this rapidly developed, larger body size and velum may be developmentally unstable, or leave other aspects of larval morphology underdeveloped at hatching (DeWitt et al., 1998; Oyarzun and Strathmann, 2011). This may be likely given the small degree of body size difference reported here. Regardless, these data suggest that larval hatching parameters such as time to and size at hatching may be plastic and responsive to environmental conditions such as predator abundance.

Capsule Morphometrics: Height and Width

In experiments from both 2010 and 2011, capsule height and width increased with maternal size. It is unclear whether this increase is simply physiological, with larger mothers having larger reproductive tracts and thus forming larger capsules and eggs, or whether larger size induces mothers to invest slightly more eggs in each capsule while expanding the volume of each egg capsule. Such a change could potentially pose problems for larvae developing in the center of the capsule if the oxygen gradient is particularly steep (Cohen and Strathmann, 1996). However, the large increase in capsule volume make it more likely that oxygen flow may be increased in these larger capsules, potentially increasing the competence of larvae to grow and develop. Alternatively, the increase in volume could be an adaptive response of large snails to general predation pressures. By increasing the volume of the capsule relative to the number of eggs inside, large snail egg capsules may be less appealing as a prey item and therefore less likely to be preyed upon.

Capsule Morphometrics: Spine Length Plasticity and Predation Trials

In trials where mud snails were exposed to green crabs, spine length and the ratio of spine length to height increased significantly in all but medium-sized snails in 2010. Indeed, changes in spine length were tightly correlated with changes in the ratio of spine length to height, suggesting that the changes induced in capsule spines did not result in related changes in capsule height, and were not scaled isometrically. This study is one of only a small number to demonstrate maternally induced defenses in offspring egg capsules, where defenses are most frequently the result of changes in the thickness of the capsule wall or by the deposition of chemical deterrents (Rawlings, 1994; Johnson and Willows, 1999). Although hermit crabs are able to prey upon egg capsules of all spine lengths, the preference for small-spined egg capsules may come from the increased handling time associated with long-spined capsules. When

attempting to remove capsules from eel grass, hermit crabs will primarily position themselves above an egg capsule and attempt to pull the capsule from the substrate (Schwab and Allen, personal observation). Because capsule spines project from the apex of each capsule, they may deter hermit crabs from positioning themselves directly above a capsule, thus increasing handling time and the potential for stationary hermit crabs to be preyed upon.

It may seem problematic that this spine-length response was mediated by green crab cues, while its adaptive function was tested with hermit crabs. Earlier studies have demonstrated that, when exposed to heightened hermit crab cue, mud snails lay egg capsules with longer spines (Santoni and Allen, 2009). While I tested the preferences of both hermit crabs and green crabs for capsules with different-sized spines, only the hermit crab appeared to forage selectively. Not surprisingly given their large size relative to egg capsules, green crabs appeared to feed indiscriminately, removing and feeding on several capsules at a time regardless of spine length (Schwab and Allen, personal observations). Thus, the response to green crab cues, despite the inability of egg capsules to mitigate green crab predation, may be the result of similar cues between green crabs and hermit crabs and the inability to distinguish the differences between these cues by mud snails (further discussion of this below).

Response of Small Snails to Urchins

It is unclear why small snails responded so strongly to green urchins. *S. droebachiensis* was originally selected as a metabolic control because it does not share a habitat with *I. obsoleta*, and is primarily herbivorous (Himmelman and Steele, 1971). Further, laboratory predation trials exposing large and small snails to the variegated urchin, *Lytechinus variegatus*, and *S. droebachiensis* resulted in no predation-related mortality (Abdel-Raheem and Boyle, unpublished results).

If these results are not the result of predation pressure, it is possible that, where mud snails and green urchins do overlap, small mud snails can suffer non-consumptive effects by green urchins. For example, in predation trials, the tube feet of urchins frequently became attached to the shells of mud snails, rendering these snails immobile until they were released or fell off the tube feet (Abdel-Raheem and Boyle, unpublished results). This immobility may translate to a significant loss in foraging time, or render mud snails more vulnerable to predation from other sources. It is presently unknown how mud snails become dislodged from tube feet, but it seems likely that snail size and weight may positively correlate with likelihood of escape. If this is so, it seems reasonable that smaller mud snails might suppress egg deposition in order to escape from urchins. However, this does not explain the strong result where spine length increased in small snails exposed to urchins. As the data presented here suggest that increased spine length is a response to predator cues, it is unlikely to also be a non-consumptive response.

Alternatively, green urchins may have historically preyed upon mud snails (despite the laboratory evidence to the contrary; Abdel-Raheem and Boyle, unpublished results), and the responses observed here are the result of evolved responses to former predation pressures. Along the northeastern Atlantic, mud snails have been driven from their historic range due to competition with periwinkles (Brenchley and Carlton, 1983). Further, urchin populations in this range have been decimated, and are only slowly recovering (Steneck et al., 2004). Therefore, the present distribution of mud snails and urchins may not reflect previous distributions where these species may have overlapped.

Because sea urchins and predaceous sea stars are so closely related, it seems feasible that the plasticity observed in this study may be the result of snails responding to similar kairomones between *S. droebachiensis* and sea stars such as *Asterias forbesii* (Atema and Burd,

1975). One of the limits to the functionality of predator-induced plasticity can be the accuracy and specificity with which prey species identify the presence of their predators (DeWitt et al., 1998). For example, when raised in the presence of closely-related molluscivorous or non-molluscivorous sunfish species, the freshwater snail, *Physella virgata*, plastically responds to both types of sunfish by reducing growth and developing rounded shells, which may reduce the likelihood of being preyed upon (Langerhans and DeWitt, 2002). Inducing these defenses when unneeded is likely energetically costly for *P. virgata*, and may relate to the responses seen in *I. obsoleta*. If this is so, then the responses observed here are not ecologically meaningful, and simply represent the induced responses that mud snails show to predators such as the green crab and sea stars. However, because the responses in question were only expressed in small snails, it seems unlikely that this would be a general response to the threat of predation by sea stars. Since sea stars prey upon snails by inserting their stomach into the shell aperture, it seems more likely that these responses would be expressed by large-bodied snails, which have the largest apertures (Santoni and Allen, unpublished results).

Once again, it seems unlikely that *S. droebachiensis* would have preyed upon mud snails or their egg capsules, as sea urchins are primarily herbivorous and detritivorous (Ruppert et al., 2004; Himmelman and Steele, 1971). However, there is at least one recorded observation of *S. droebachiensis* feeding on the egg capsules of a gastropod. Similar to *I. obsoleta*, the Pribilof whelk, *Neptunea pribiloffensis*, lays large egg masses with individually encapsulated embryos on the benthos of the intertidal, which are frequently preyed upon by *S. droebachiensis*. Interestingly, *N. pribiloffensis* also defends its egg capsules, though it does so by depositing them near groups of sea anemones, which protect capsules from predation by sea urchins and other intertidal predators (Shimek, 1981). Additionally, there is at least one dense population of *S.*

droebachiensis inhabiting the sandy subtidal in the Gulf of Maine, which may overlap with mud snail habitat (Harris, unpublished observations). At this high density, it seems likely that the urchins on this sandflat utilize mud snails or their egg capsules as a food resource. Although there are presently no observations of green urchins preying upon mud snail egg capsules in the laboratory, these observational data suggest that urchins at least have the capacity to feed on egg capsules in the field. If so, the choice of green urchins as a metabolic control was an unwitting mistake, and should be treated as a second predator in this study.

CONCLUSION

I investigated the various ways that maternal effects on reproduction in *Ilyanassa obsoleta* can be modulated by maternal size, predator presence, and the interaction between the two. Over the course of two field seasons, I confirmed that (1) large snails do invest more in reproduction than small snails; (2) that large snails laid larger egg capsules than small snails; (3) that snails do induce the formation of elongated capsule spines in the presence of *C. maenas*; (4) that snails hatched sooner and at a smaller size in the presence of *C. maenas*. However, I found little evidence that *C. maenas* induces snails to invest less in reproduction; rather, reproductive investment was primarily affected by maternal size. Altogether, these results suggest that maternal effects in species such as *I. obsoleta* can be size- and context-dependent, can play an important role in defending embryos from predators during early development, and may persist post-hatching.

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FIGURES AND TABLES

FIGURES

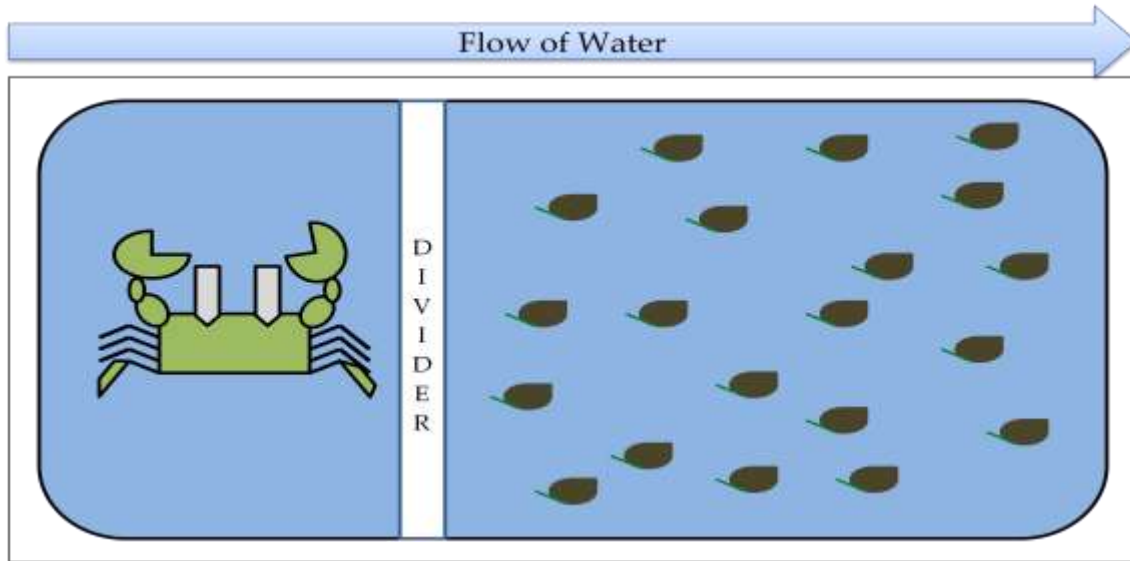


Figure 1. Experimental unit. For all experiments, 20 snails were separated on one side of an asymmetric and porous divider, on which snails generally laid capsules. On the other side of this divider, a predator such as the green crab was isolated. Flow-through seawater allowed predator kairomones to be transmitted to the snails on the other side of the divider.

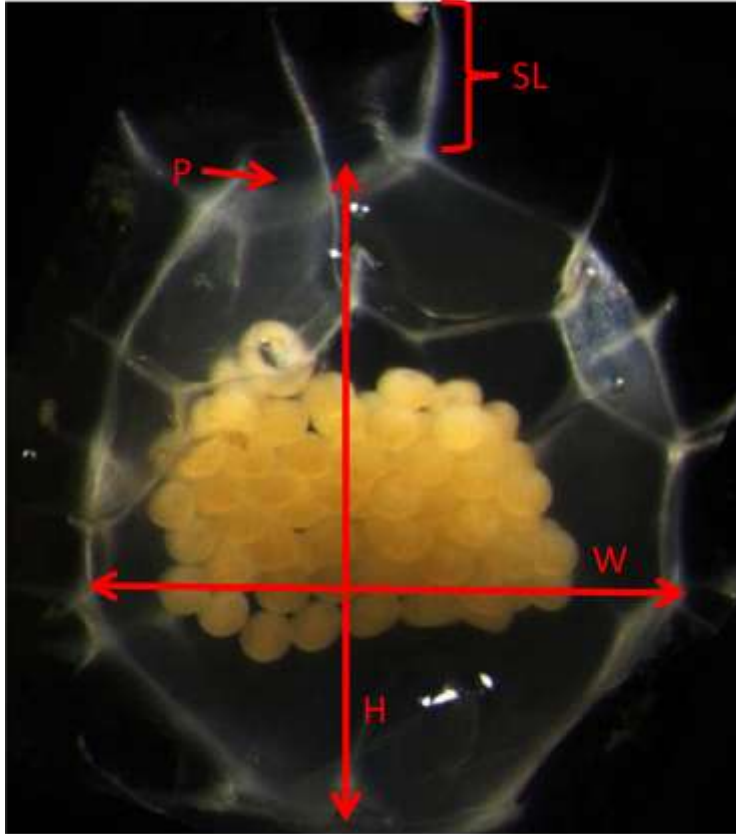


Figure 2. Metrics for capsule morphology analysis. Capsule height (H) was measured using the longest distance from the capsule base to the top of the capsule plug (P). Capsule width (W) was defined as the furthest two points perpendicular to H. The longest capsule spine was used in a measure of spine length (SL), measured from the capsule wall to the tip of the spine.



Figure 3. Sex determination in *Ilyanassa obsoleta*. (A) The female gonad is characterized by a cream to light-orange coloration at the apex of the snail body, as well as a granular appearance indicative of ovarioles. (B) In addition to its characteristic gonad morphology, females possess a milky-white egg capsule gland located on the right side of the visceral mass and mantle cavity (circled in white). (C) The male gonad is characterized by a dark-orange to red coloration at the apex of the snail body, as well as enlarged seminal vesicles during the reproductive season (indicated by the white arrow). Pictures taken on a dissecting microscope at 0.8x magnification for A and C and 1.25x magnification for B.

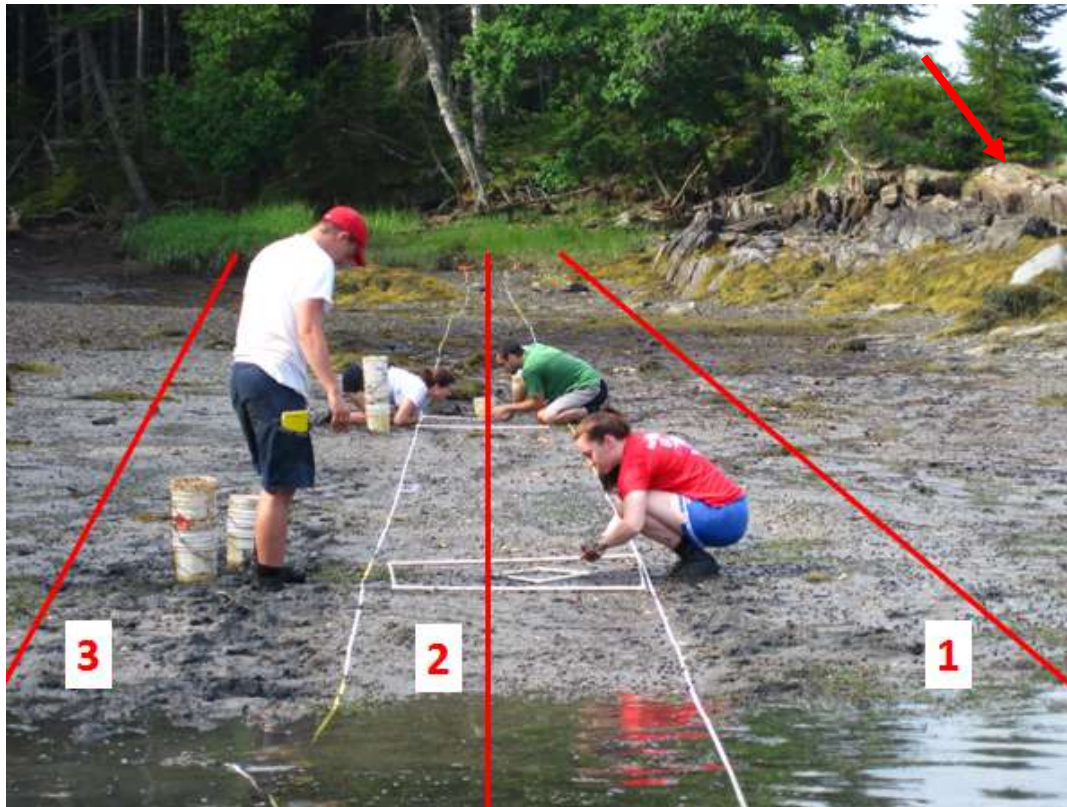


Figure 4. Using transects to analyze mudflat variation in snail density, size, sex ratio, and biomass. Three transects were utilized in order to track these variables vertically and horizontally across the mudflat. Transects were sampled using 1 m² quadrats with 0.25 m² quadrats for sub-sampling. Green crabs inhabit the rocky section of the intertidal, located nearest transect 1 (red arrow), and extending out of frame.

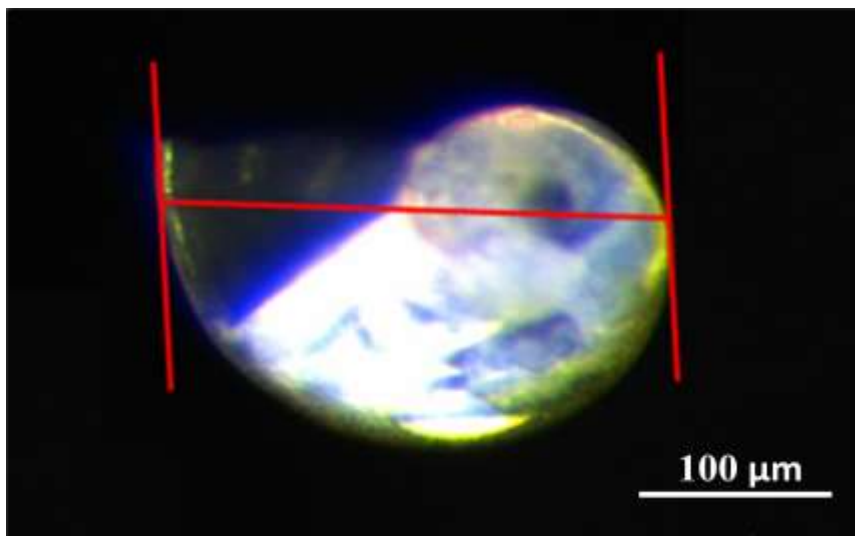


Figure 5. Measuring larvae at hatching. Larvae were fixed immediately upon hatching and a single measure (body size) was taken from the two furthest points across the body wall. An example measurement is shown in red.



Figure 6. Experimental unit for predation trials. 10 small- ($0 - 375 \mu\text{m}$) and large-spined ($575 \mu\text{m}$ or greater) capsules were randomly assorted on a blade of eelgrass. Predators (green crab, urchin, and hermit crab) were placed inside the experimental apparatus and allowed to prey upon approximately 10 capsules. Egg capsules are circled in red.

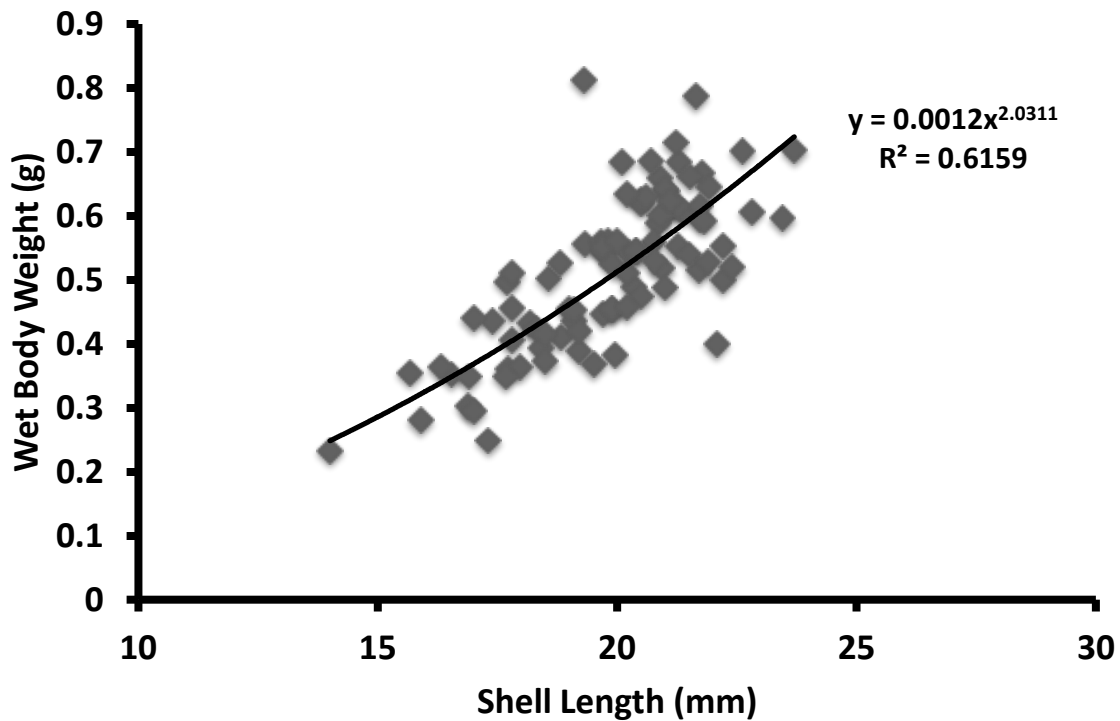


Figure 7. Shell length (mm) regressed with wet body weight (g). Shell length explains approximately 62% of the variation in wet body weight.

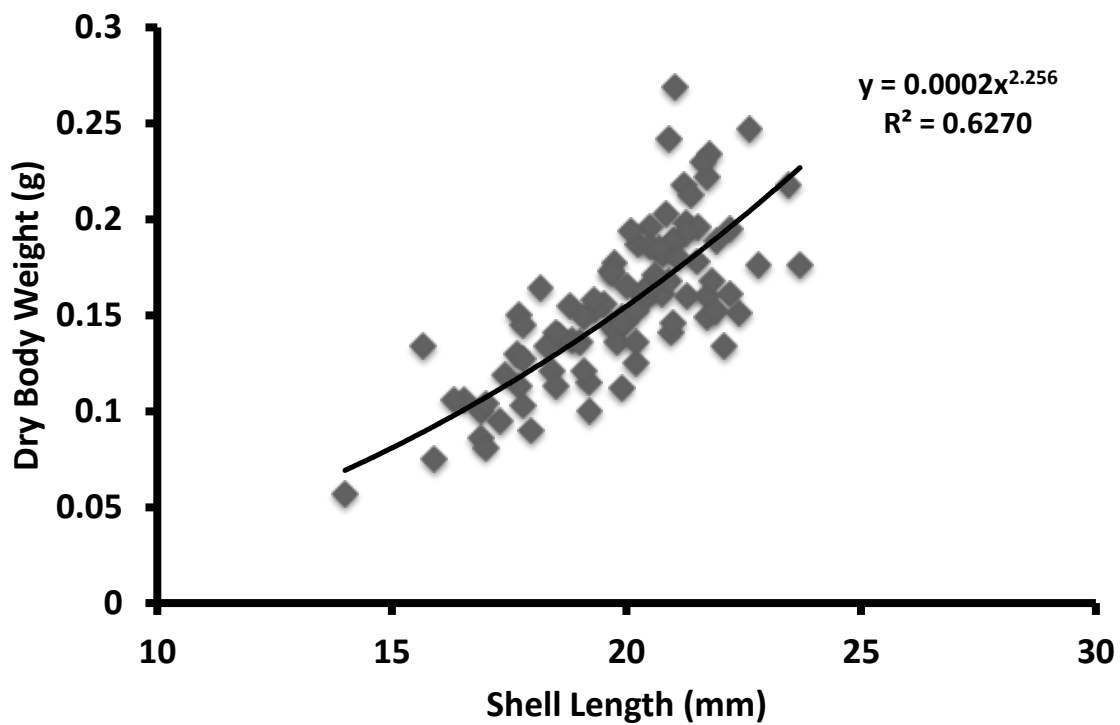


Figure 8. Shell length (mm) regressed with dry body weight (g). Shell length explains approximately 63% of the variation in dry body weight.

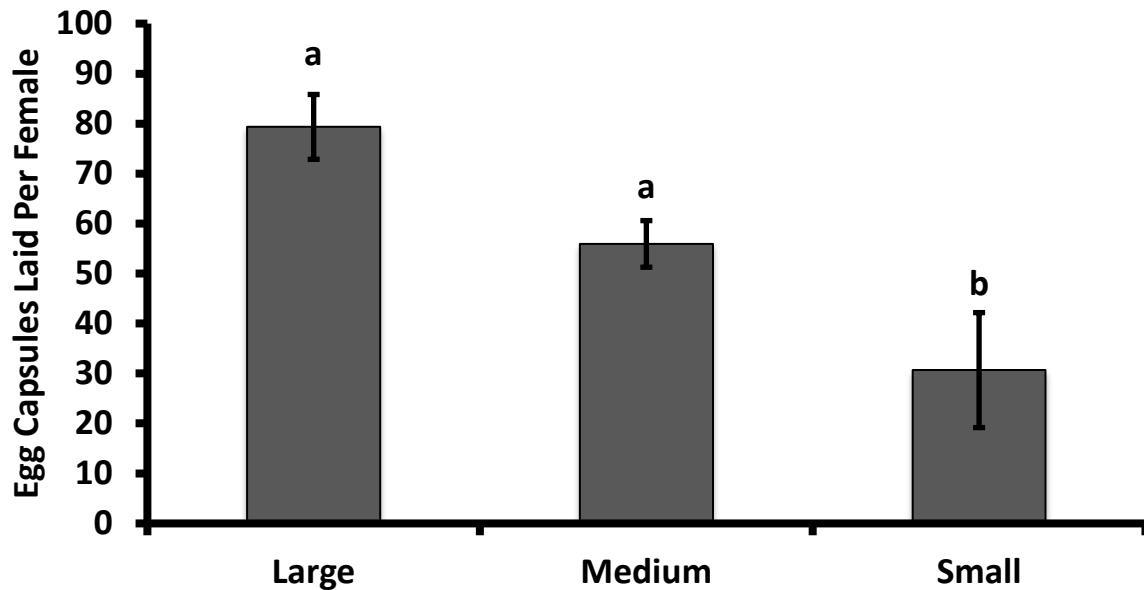


Figure 9. Total egg capsule output per female by size class. Large snails laid significantly more egg capsules ($\bar{x} = 79$ capsules) than small snails ($\bar{x} = 31$ capsules), but not medium snails ($\bar{x} = 56$ capsules). Further, there was no difference between medium and small size classes. Error bars represent the standard error; letters indicate statistically significant groups.

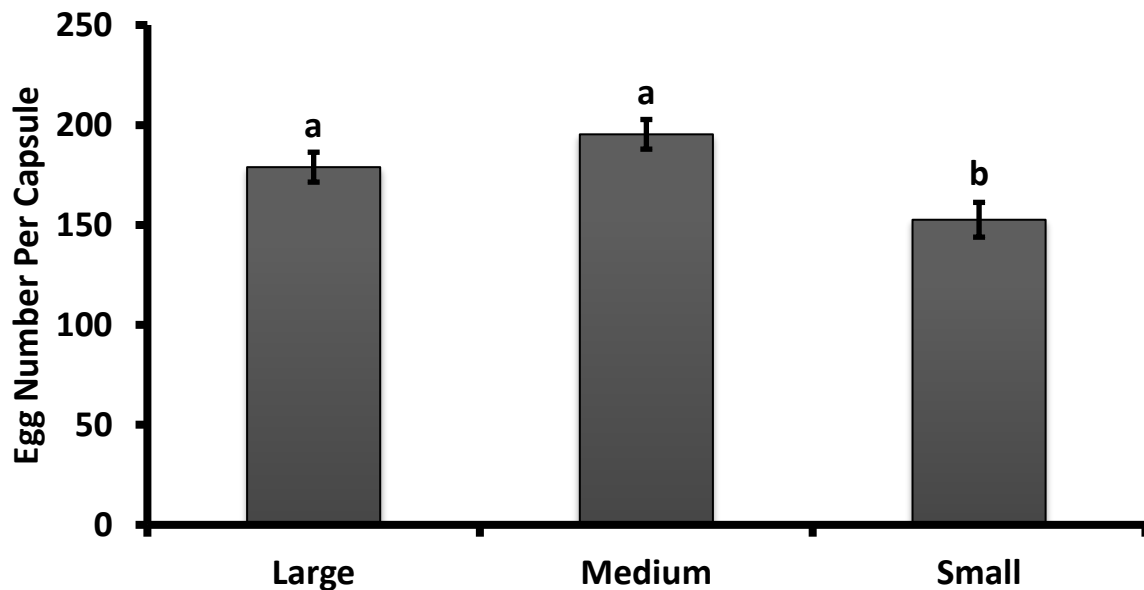


Figure 10. Egg number per capsule by snail size class. Large ($\bar{x} = 179$ eggs) and medium snails ($\bar{x} = 195$ eggs) deposited more eggs in their capsules than small snails ($\bar{x} = 153$ eggs). However, there was no difference in egg number between large and medium size classes. Error bars represent the standard error; letters indicate statistically significant groups.

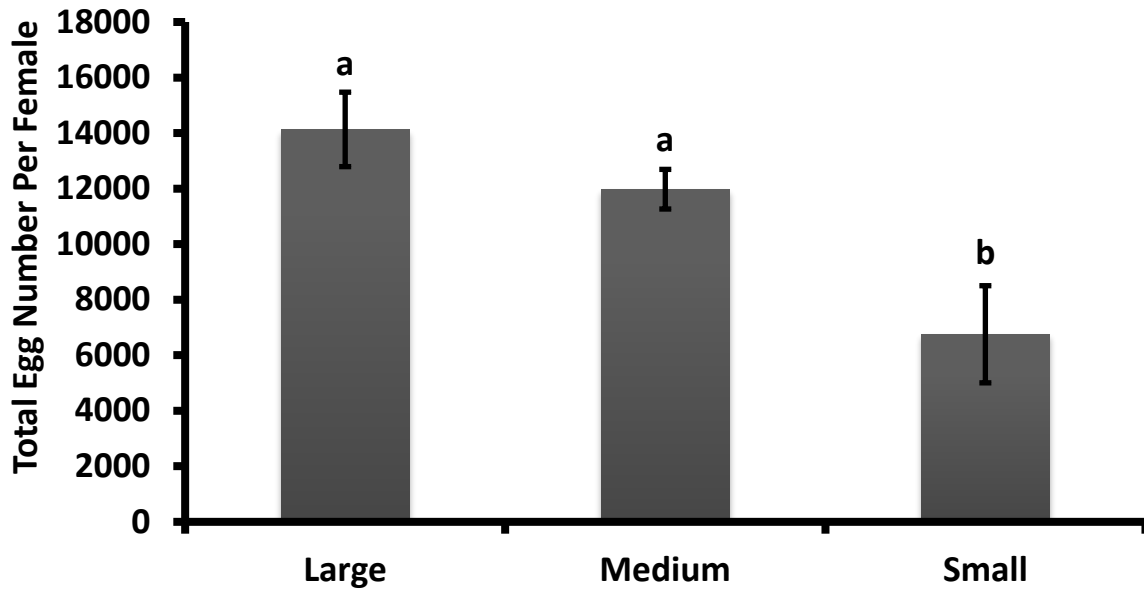


Figure 11. Total reproductive output (product of egg number and capsule number) expressed as egg number by size class. Large snails ($\bar{x} = 14134$ eggs) and medium snails ($\bar{x} = 11980$ eggs) laid significantly more eggs than small snails ($\bar{x} = 6754$ eggs). However, there was no difference between the reproductive output of large and medium size classes. Error bars represent the standard error; letters indicate statistically significant groups.

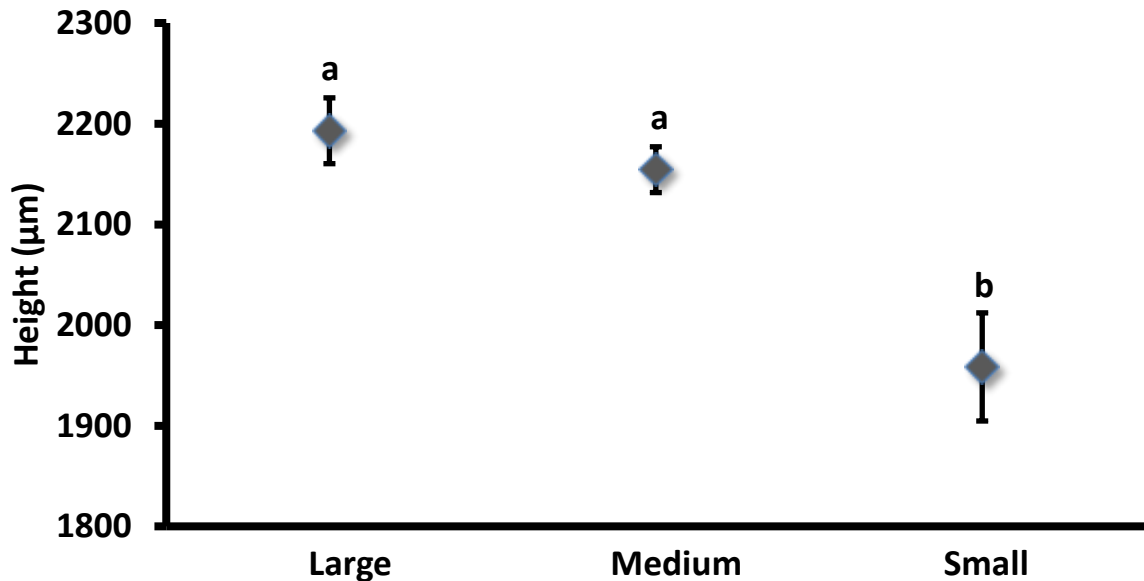


Figure 12. Egg capsule height by snail size class. Large snails ($\bar{x} = 2193$ µm) and medium snails ($\bar{x} = 2154$ µm) laid significantly taller egg capsules than small snails ($\bar{x} = 1958$ µm). However, there was no difference between the capsule height of large and medium size classes. Error bars represent the standard error; letters indicate statistically significant groups.

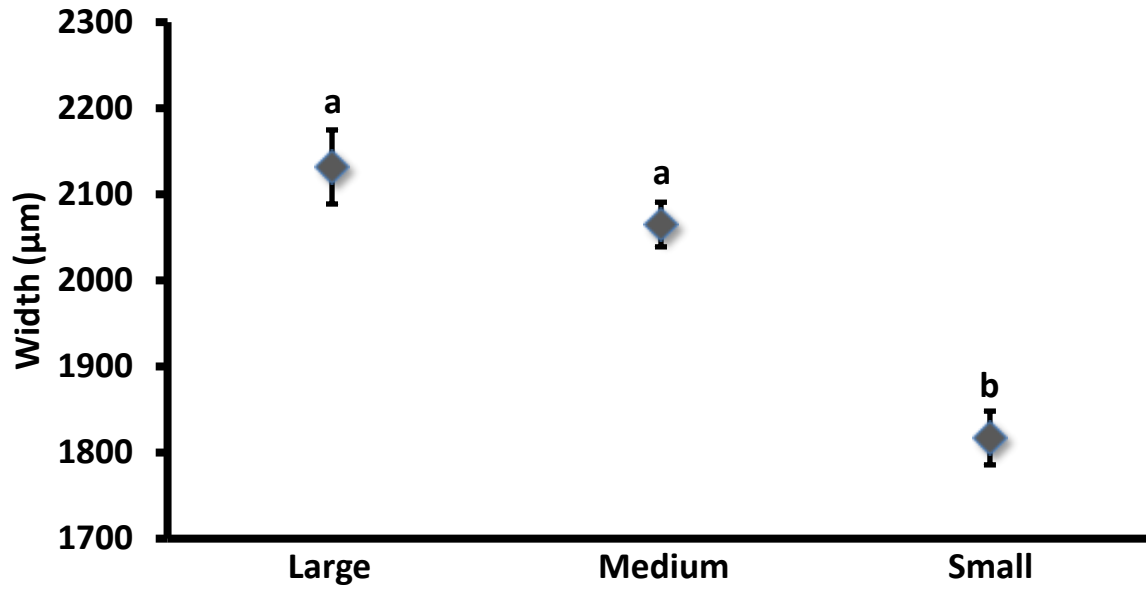


Figure 13. Egg capsule width by snail size class. Large snails ($\bar{x} = 2132 \mu\text{m}$) and medium snails ($\bar{x} = 2065 \mu\text{m}$) laid significantly wider egg capsules than small snails ($\bar{x} = 1817 \mu\text{m}$). However, there was no difference between the capsule width of large and medium size classes. Error bars represent the standard error; letters indicate statistically significant groups.

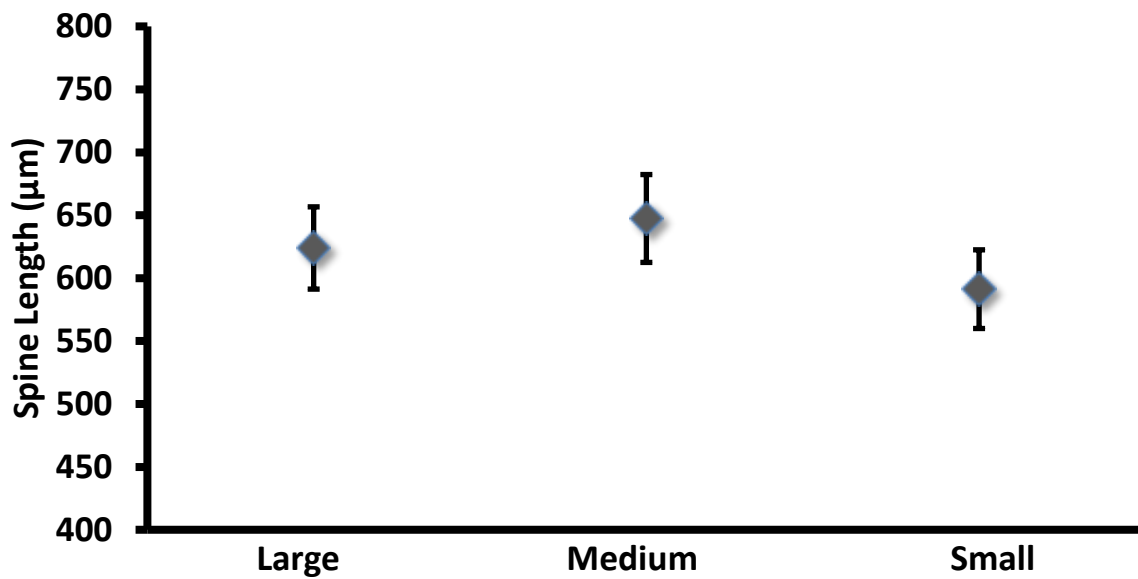


Figure 14. Egg capsule spine length by snail size class. There was no significant difference between large ($\bar{x} = 624 \mu\text{m}$), medium ($\bar{x} = 647 \mu\text{m}$), or small ($\bar{x} = 551 \mu\text{m}$) snails in the length of capsule spines. Error bars represent the standard error.

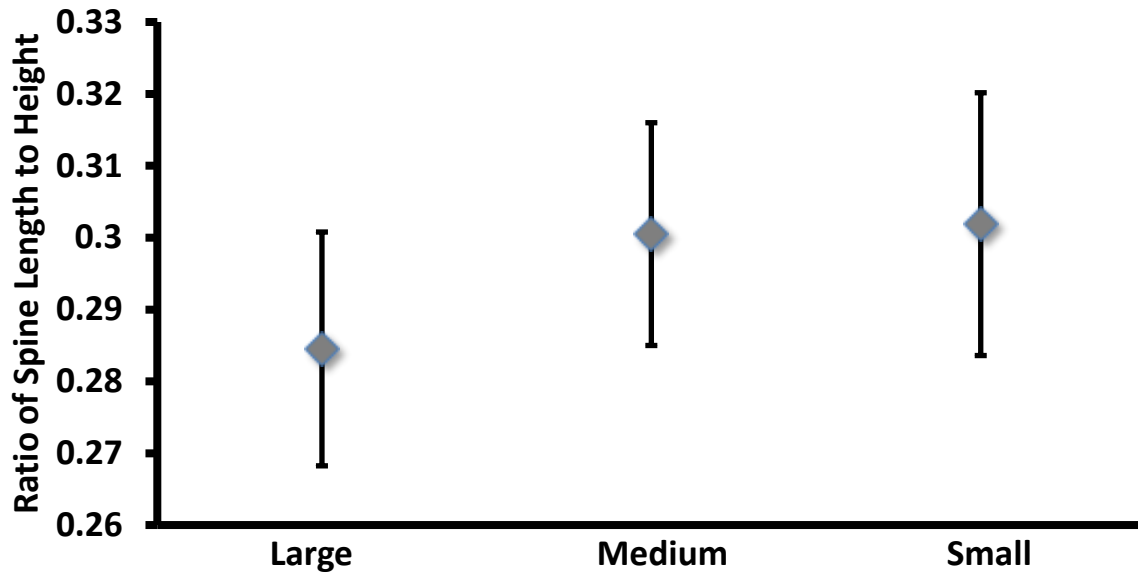


Figure 15. Ratio of spine length to height. There was no significant difference between large ($\bar{x} = 0.28$), medium ($\bar{x} = 0.30$), or small ($\bar{x} = 0.30$) snails in the ratio of capsule spines to height. Error bars represent the standard error.

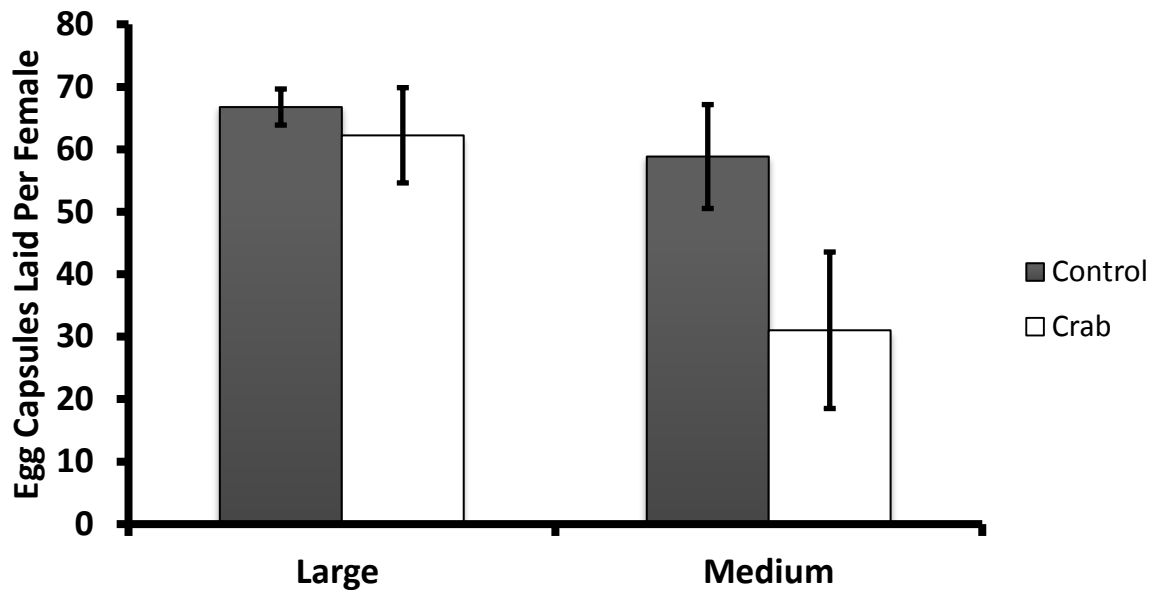


Figure 16. Total egg capsule output per female by snail size class and treatment group. Large snails laid significantly more egg capsules per female ($\bar{x} = 64$ capsules) than medium snails ($\bar{x} = 45$ capsules). However, there was no difference between crab ($\bar{x} = 51$ capsules) and control ($\bar{x} = 63$ capsules) treatments. Error bars represent the standard error.

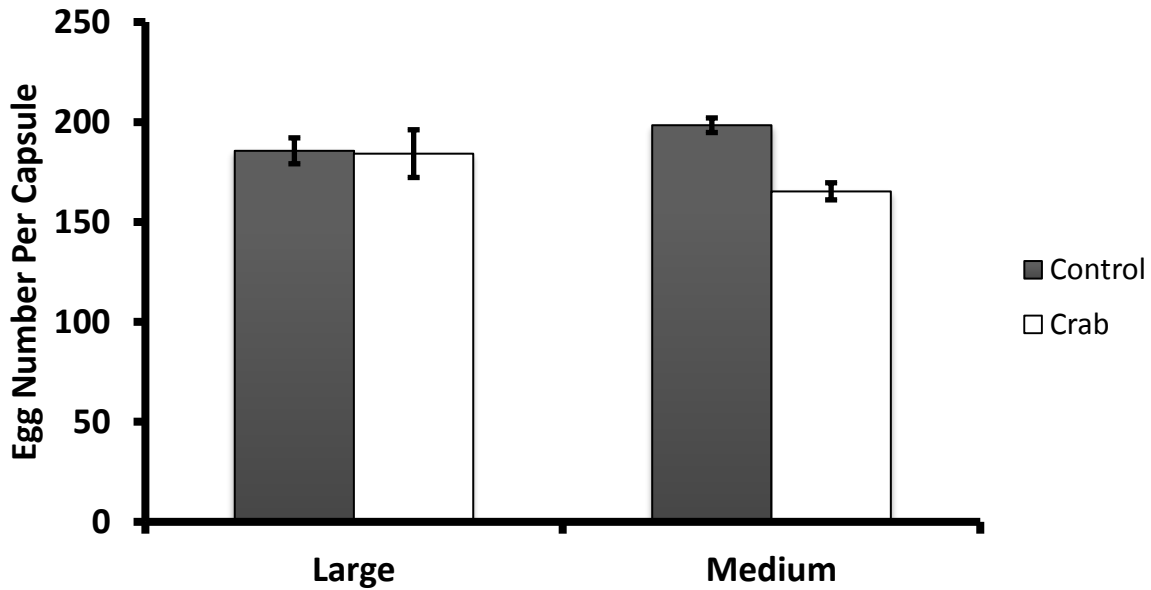


Figure 17. Egg number per capsule by size class and treatment. There was no difference in egg number between large ($\bar{x} = 185$ eggs) and small snails ($\bar{x} = 182$ eggs), and crab ($\bar{x} = 177$ eggs) and control ($\bar{x} = 191$ eggs) treatments. Error bars represent the standard error.

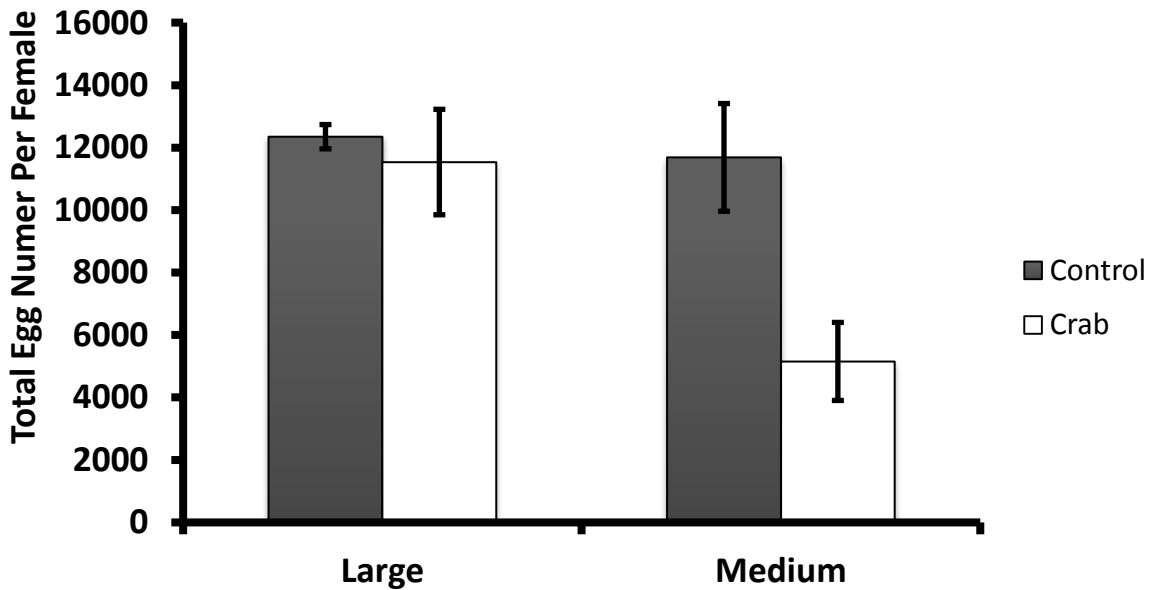


Figure 18. Total reproductive output (product of egg number and capsule number) as egg number by size class and treatment group per female. There was no difference in the total number of eggs produced between large ($\bar{x} = 11896$ eggs) and medium snails ($\bar{x} = 8418$ eggs); however, in the presence of crabs, snails depressed the total number of eggs produced ($\bar{x} = 9141$ eggs) as compared to the control treatment ($\bar{x} = 12064$ eggs). Error bars represent the standard error.

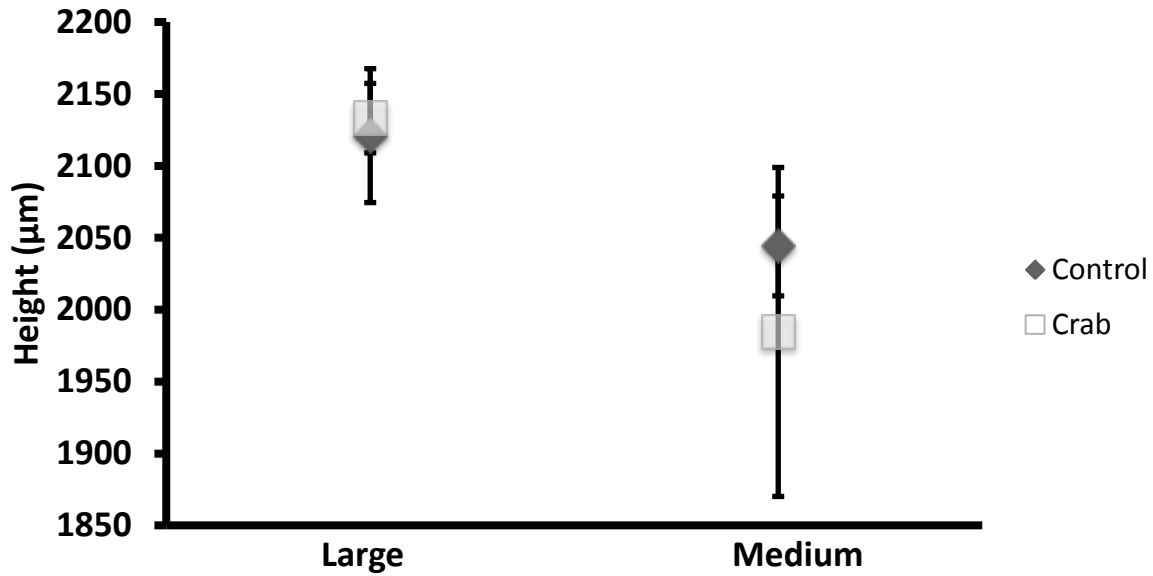


Figure 19. Egg capsule height by snail size class and treatment group. Egg capsule height was not significantly different between large ($\bar{x} = 2127 \mu\text{m}$) and medium snails ($\bar{x} = 2014 \mu\text{m}$). Further, there was no difference in capsule height between crab ($\bar{x} = 2059 \mu\text{m}$) and control ($\bar{x} = 2083 \mu\text{m}$) treatments. Error bars represent the standard error.

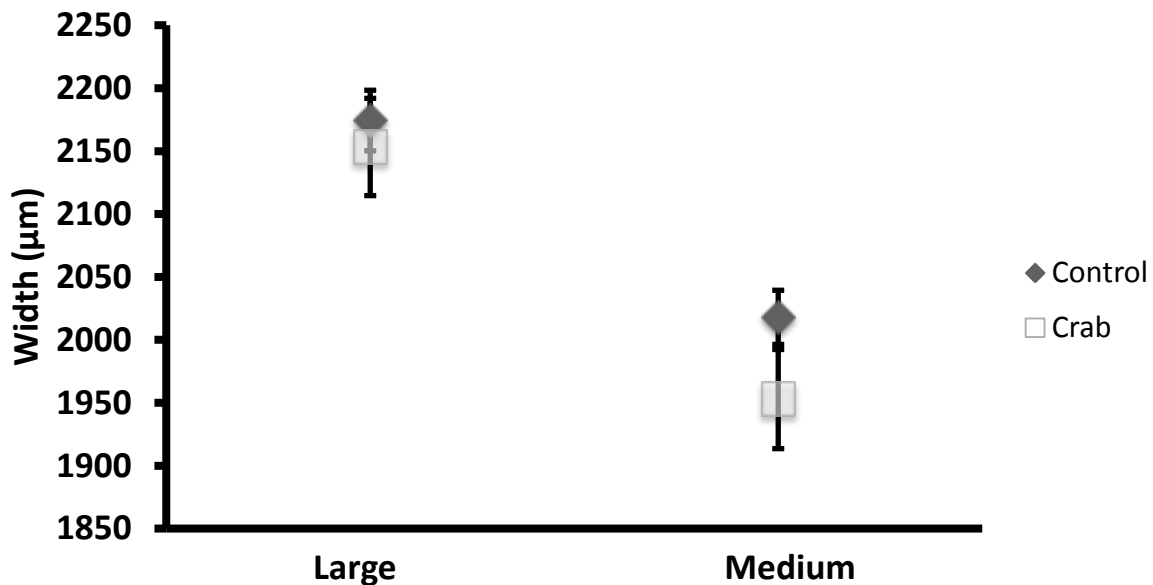


Figure 20. Egg capsule width by snail size class and treatment group. Egg capsule width was significantly different between large ($\bar{x} = 2163 \mu\text{m}$) and medium snails ($\bar{x} = 1985 \mu\text{m}$). However, there was no difference in capsule width between crab ($\bar{x} = 2053 \mu\text{m}$) and control ($\bar{x} = 2096 \mu\text{m}$) treatments. Error bars represent the standard error.

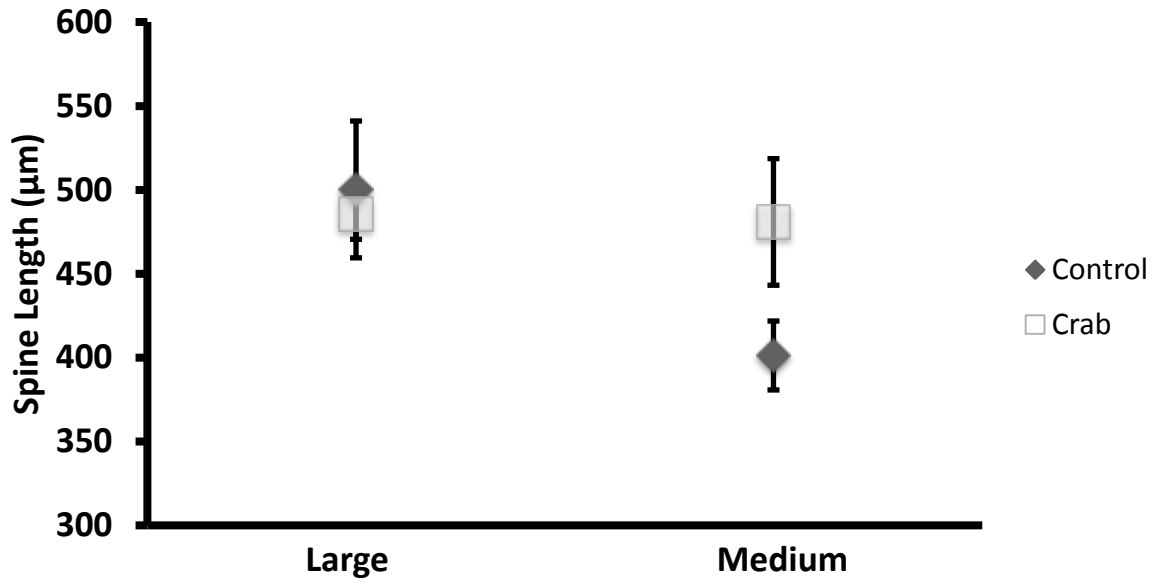


Figure 21. Egg capsule spine length by snail size class and treatment group. Egg capsule spine length was not significantly different between large ($\bar{x} = 493 \mu\text{m}$) and medium snails ($\bar{x} = 441 \mu\text{m}$). Further, there was no difference in spine length between crab ($\bar{x} = 483 \mu\text{m}$) and control ($\bar{x} = 451 \mu\text{m}$) treatments. Error bars represent the standard error.

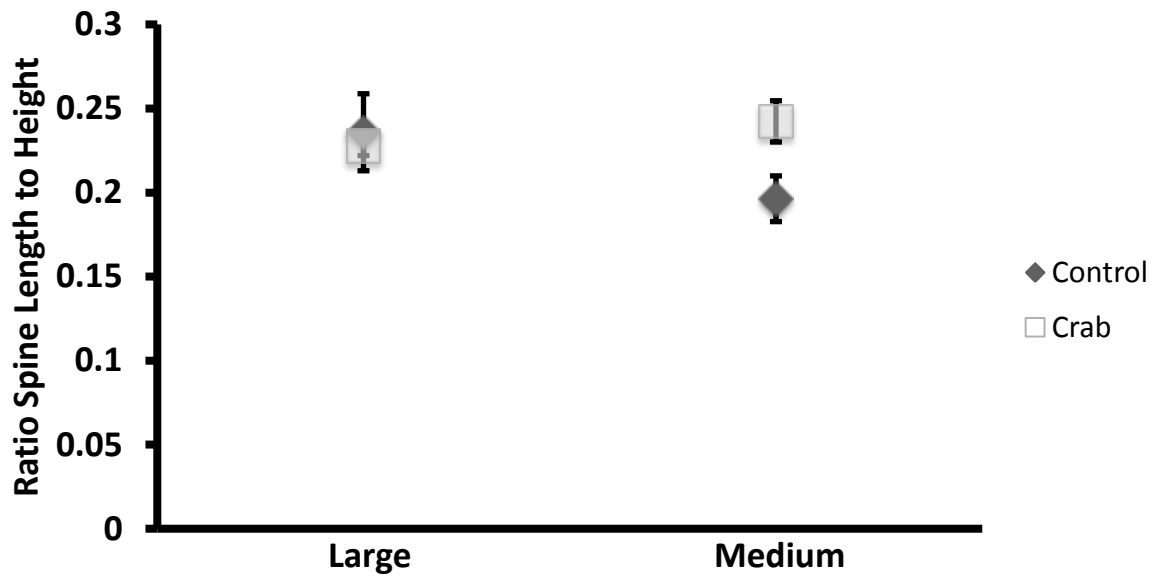


Figure 22. Ratio of spine length to egg capsule height. The ratio of egg capsule spine length to capsule height was not significantly different between large ($\bar{x} = 0.232$) and medium snails ($\bar{x} = 0.219$). Further, there was no difference between crab ($\bar{x} = 0.235$) and control ($\bar{x} = 0.216$) treatments. Error bars represent the standard error.

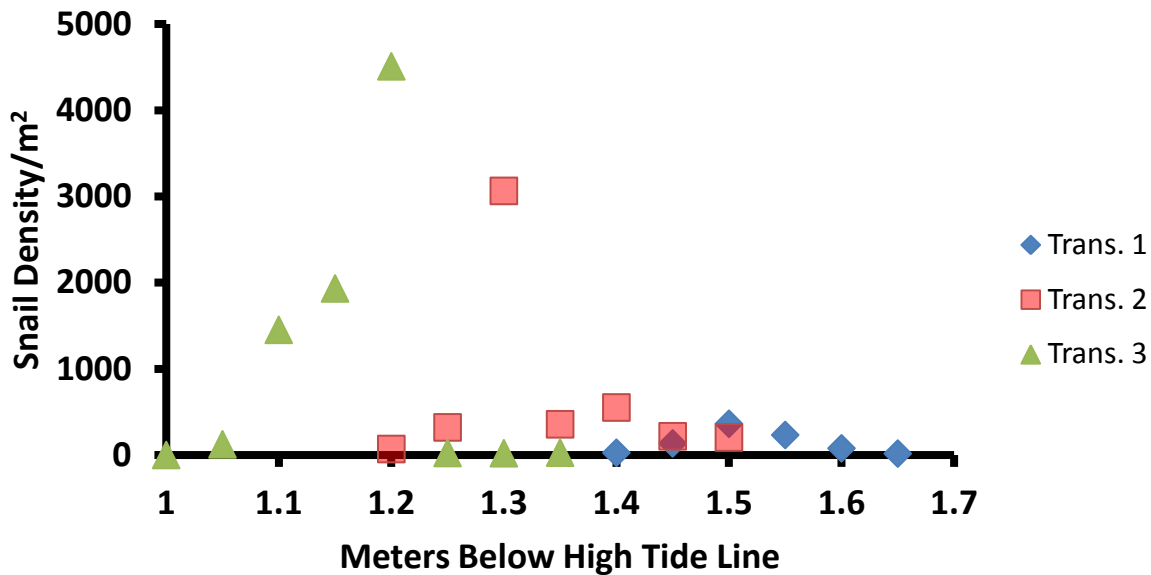


Figure 23. Snail density by transect and tidal height. Snail density varies significantly along each transect, with transect 3 (furthest from green crab habitat) containing the greatest density of snails on average, while transect 1 (nearest the green crab habitat) contains the lowest density of snails on average. This suggests that snail spatial distribution may be related to the threat of predation by green crabs. Further, because snail density appears to decrease on average with increasing tidal depth, this may be a way of avoiding the green crabs that are generally found deeper in the intertidal.

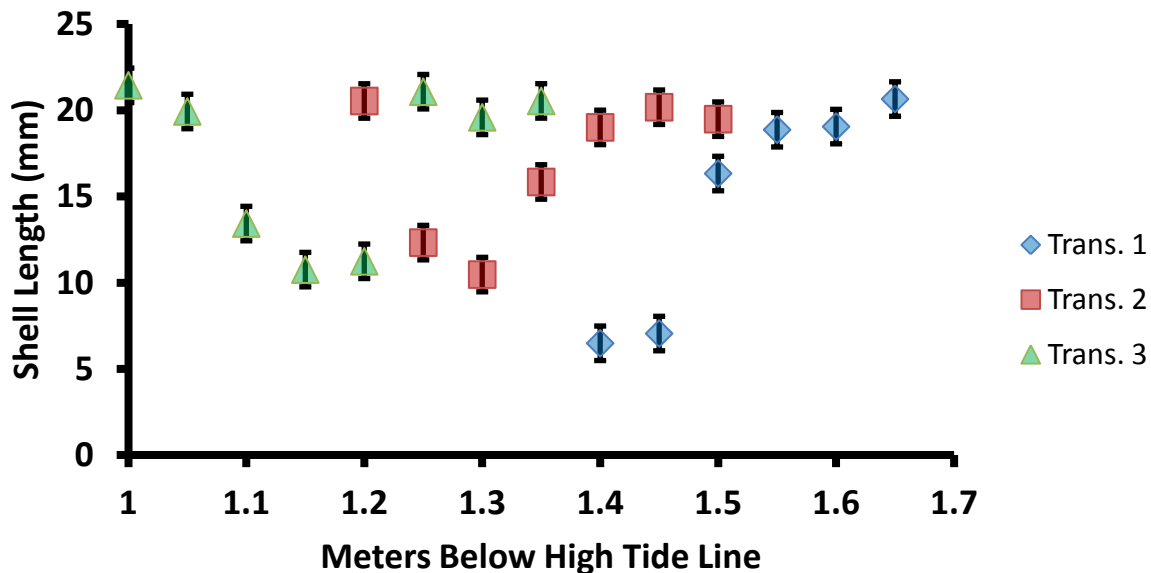


Figure 24. Shell length by transect and tidal height. The largest snails are distributed at the furthest point below the high tide line along each transect, while the smallest snails are found at the mid-point of transects 2 and 3 and the beginning of transect 1, suggesting that the largest snails may have reached a size threshold to avoid low intertidal predation by green crabs.

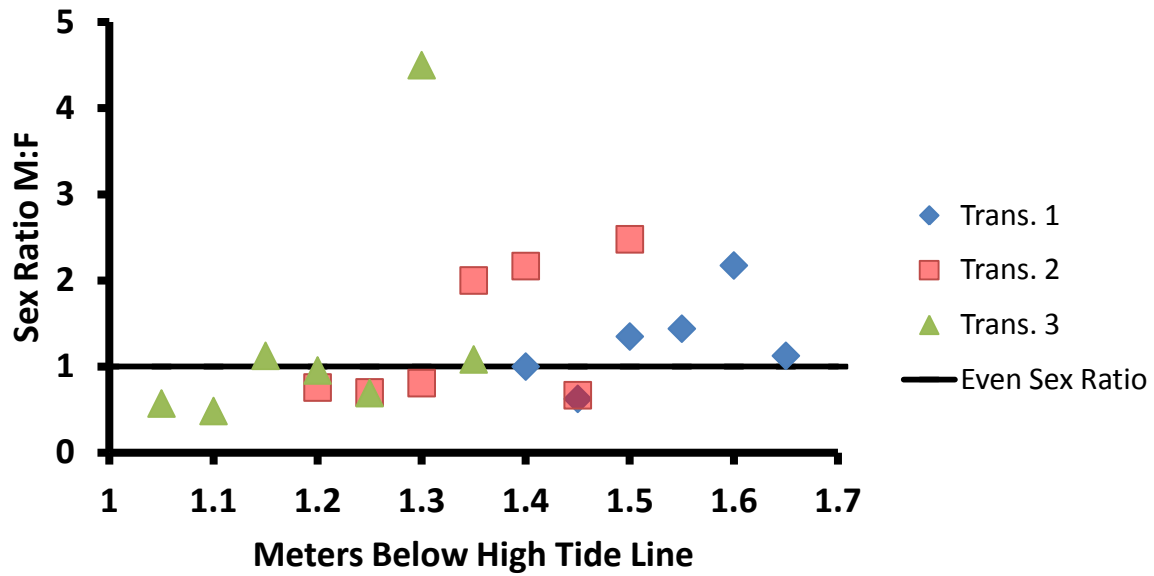


Figure 25. Sex ratio by transect and tidal height. Sex ratio varies significantly with tidal height and transect. Males and females both reach their largest proportion in transect 3, which is likely due to the increased snail density along this transect. Altogether, males seem to predominate in the population.

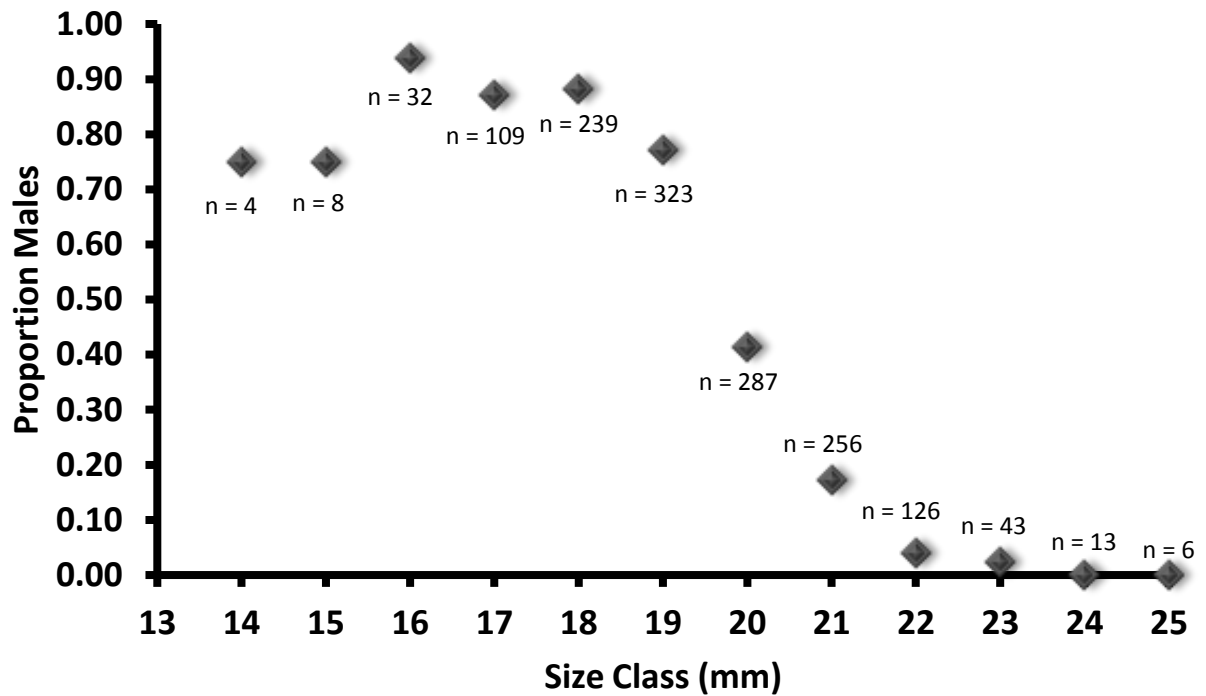


Figure 26. Proportion of males by size class. When small, snails are primarily male. At larger size classes (22 mm – 25 mm), snails are almost exclusively female. Between the 19 mm and 20 mm size classes, the proportion of males dramatically decreases from 0.77 to 0.41. Here, the 14 mm size class includes all snails with a shell length from 14.01 mm to 15.00 mm, the 15 mm size class includes all snails with a shell length from 15.01 mm to 16.00, and so on through the 25 mm size class.

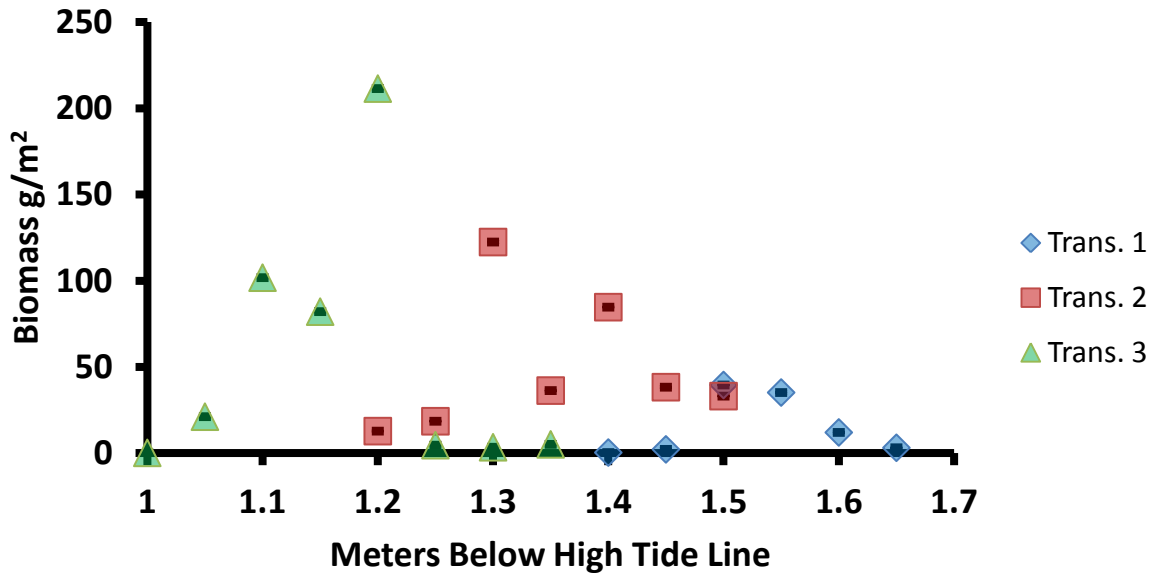


Figure 27. Biomass by transect and tidal height. Biomass varies significantly along each transect, with transect 3 (furthest from green crab habitat) containing the greatest biomass of snails on average, while transect 1 (nearest the green crab habitat) contains the lowest biomass of snails on average. Additionally, biomass appears to decrease with increased tidal depth. This suggests that snail spatial distribution may be related to the threat of predation by green crabs.

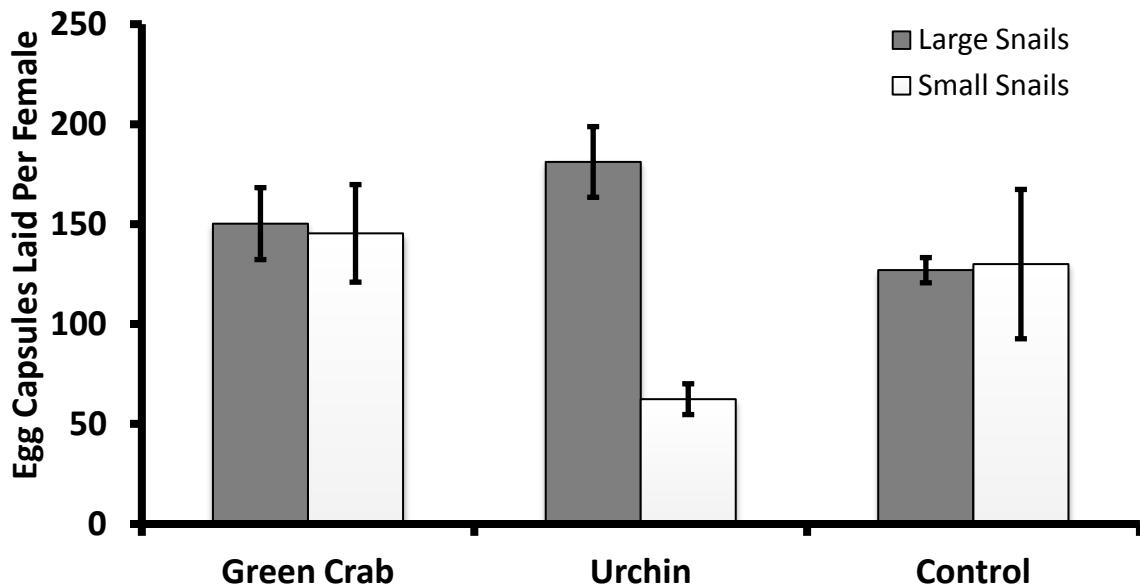


Figure 28. Total egg capsule output per female by snail size and treatment. Large snails did not lay more egg capsules ($\bar{x} = 153$ capsules) than small snails ($\bar{x} = 113$ capsules). Further, green crab ($\bar{x} = 148$ capsules), urchin ($\bar{x} = 122$ capsules), and control groups ($\bar{x} = 128$ capsules) did not lay significantly different numbers of egg capsules from one another. The significant interaction between size and treatment is driven largely by the urchin treatment, where large snails exposed to urchins lay more egg capsules than large snails in other treatments, while the converse is true for small snails. Error bars represent the standard error.

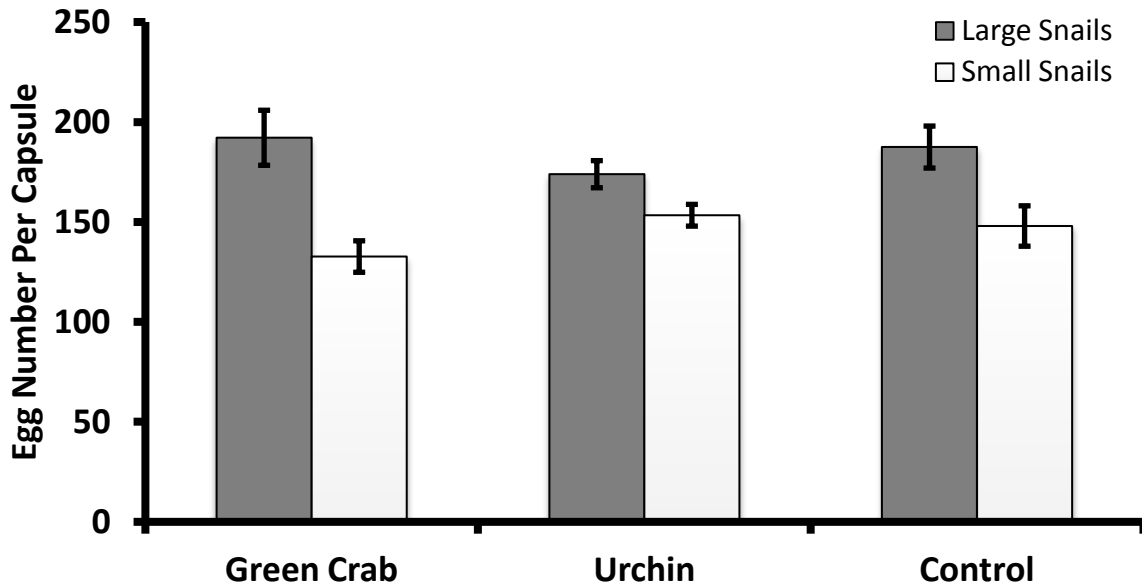


Figure 29. Number of eggs per capsule by snail size and treatment. Large snails laid significantly more eggs ($\bar{x} = 185$ eggs) than small snails ($\bar{x} = 145$ eggs). Green crab ($\bar{x} = 162$ eggs), urchin ($\bar{x} = 164$ eggs), and control groups ($\bar{x} = 168$ eggs) did not lay significantly different numbers of eggs from one another. However, there was a significant interaction between these factors, with large snails appearing to decrease and small snails appearing to increase the number of eggs per capsule in the presence of the green urchin. Error bars represent the standard error.

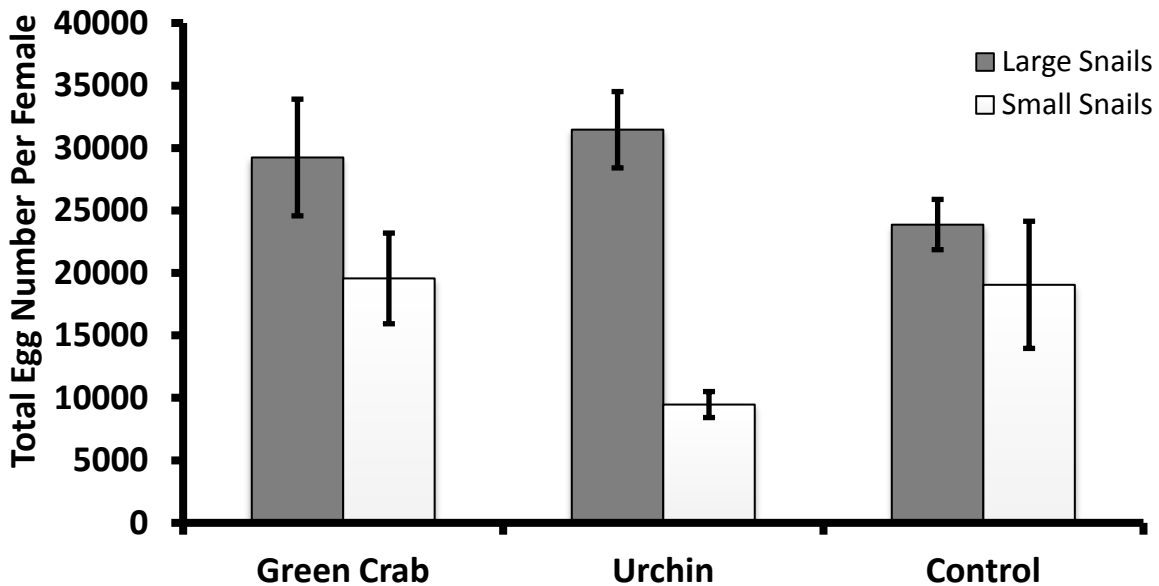


Figure 30. Total reproductive output as egg number by size class and treatment. Overall, large snails ($\bar{x} = 28186$ eggs) laid more eggs than small snails ($\bar{x} = 16016$ eggs). However, green crab ($\bar{x} = 24395$ eggs), urchin ($\bar{x} = 20456$ eggs), and control groups ($\bar{x} = 21454$ eggs) did not lay significantly different numbers of eggs from one another. Although there was no significant interaction, large snails appear to increase and small snails appear to decrease their total reproductive output in the presence of the green urchin. Error bars represent the standard error.

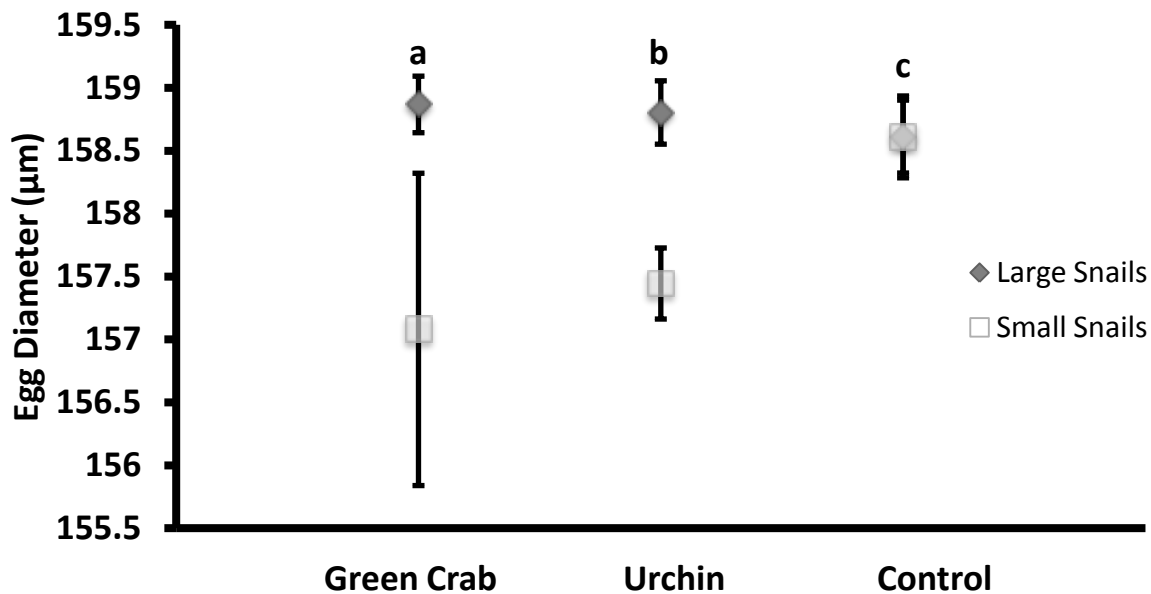


Figure 31. Egg size by snail size and treatment. Large snails laid significantly larger eggs (\bar{x} = 158.76 μm diameter) than small snails (\bar{x} = 157.71 μm diameter), though egg sizes were approximately the same between size classes in the control treatment. Additionally, green crab (\bar{x} = 157.97 μm diameter), urchin (\bar{x} = 158.12 μm diameter), and control groups (\bar{x} = 158.61 μm diameter) laid eggs of significantly different sizes from one another. Error bars represent the standard error; letters indicate statistically significant groups.

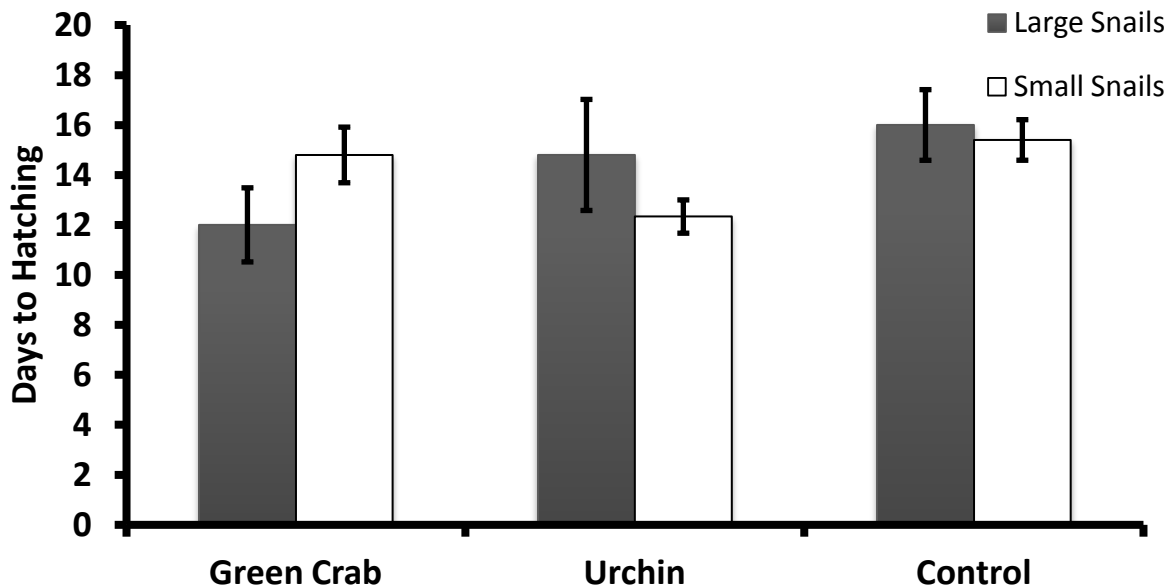


Figure 32. Larval time to hatching by maternal size and treatment. Larvae of large mothers (\bar{x} = 14.3 days) and small mothers (\bar{x} = 14.2 days) hatched at the same time. Further, there was no difference between green crab (\bar{x} = 13.4 days), urchin (\bar{x} = 13.9 days), and control larvae (\bar{x} = 15.7 days) in the time at which they hatched. However, there does appear to be a trend towards larvae exposed to green crab cue hatching earlier, at approximately 56 hours before larvae in control groups. Error bars represent the standard error.

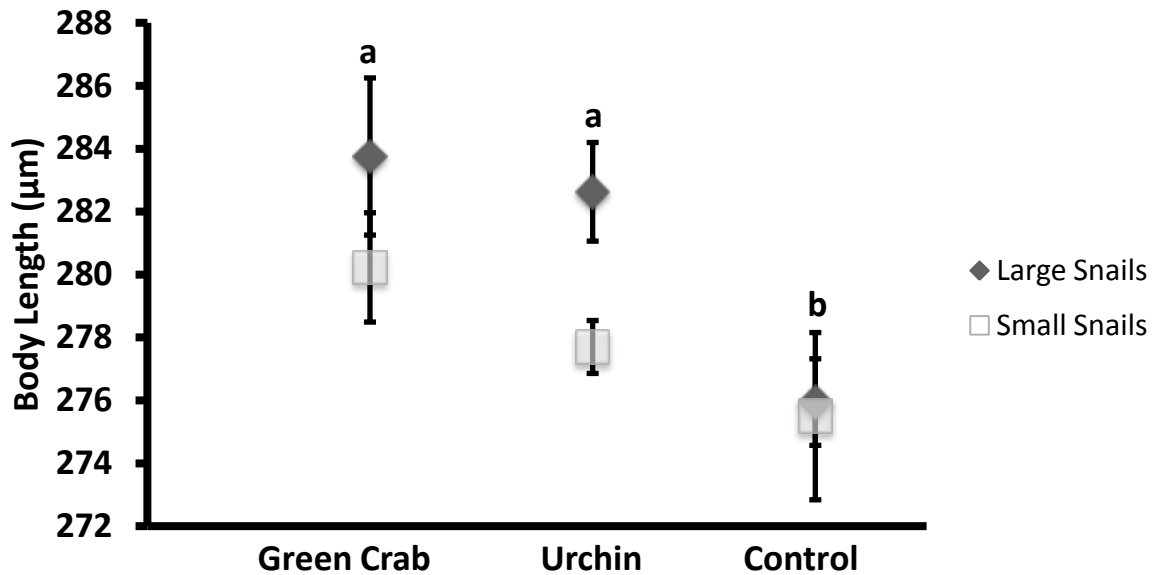


Figure 33. Larval size at hatching by size and treatment. Larvae of large mothers ($\bar{x} = 280.78 \mu\text{m}$) produced larger larvae than those of small mothers ($\bar{x} = 277.81 \mu\text{m}$). In the presence of green crab and urchin cues, larvae hatched at a significantly larger size ($\bar{x} = 281.99$ and $280.17 \mu\text{m}$; respectively) than controls ($\bar{x} = 275.72 \mu\text{m}$). Error bars represent the standard error; letters indicate statistically significant groups.

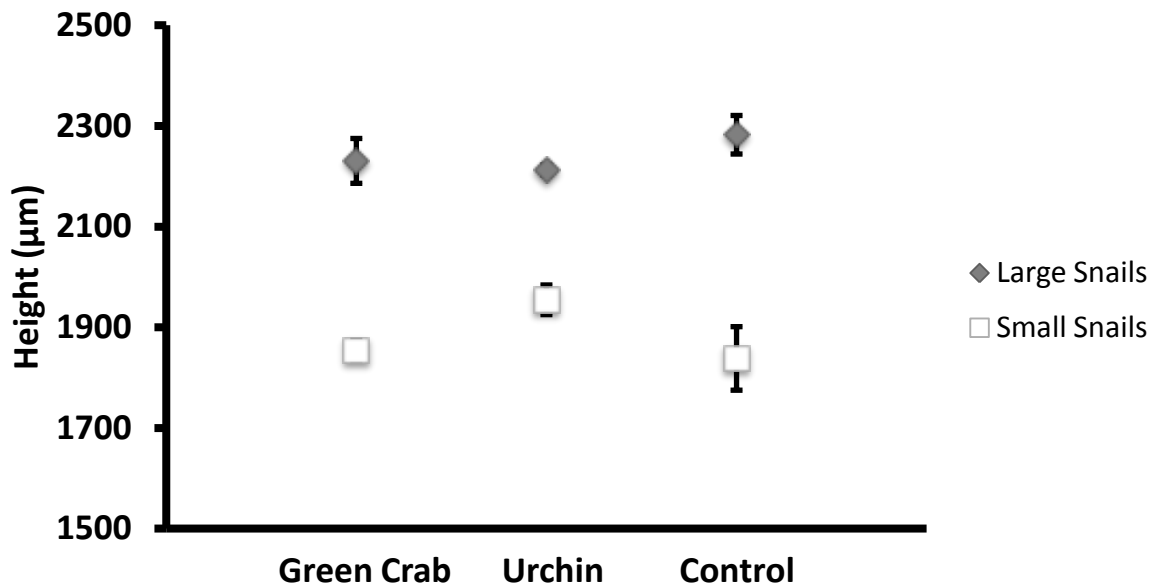


Figure 34. Capsule height by snail size and treatment. Large snails laid taller ($\bar{x} = 2242 \mu\text{m}$) egg capsules than small snails ($\bar{x} = 1882 \mu\text{m}$). There was no difference in capsule height between green crab ($\bar{x} = 2042 \mu\text{m}$), urchin ($\bar{x} = 2084 \mu\text{m}$), or control ($\bar{x} = 2060 \mu\text{m}$) treatments. Additionally, there was an interaction between snail size and treatment, likely the result of small snails in the urchin treatment increasing capsule height, while large snails exposed to this same treatment decreased capsule height. Error bars represent the standard error.

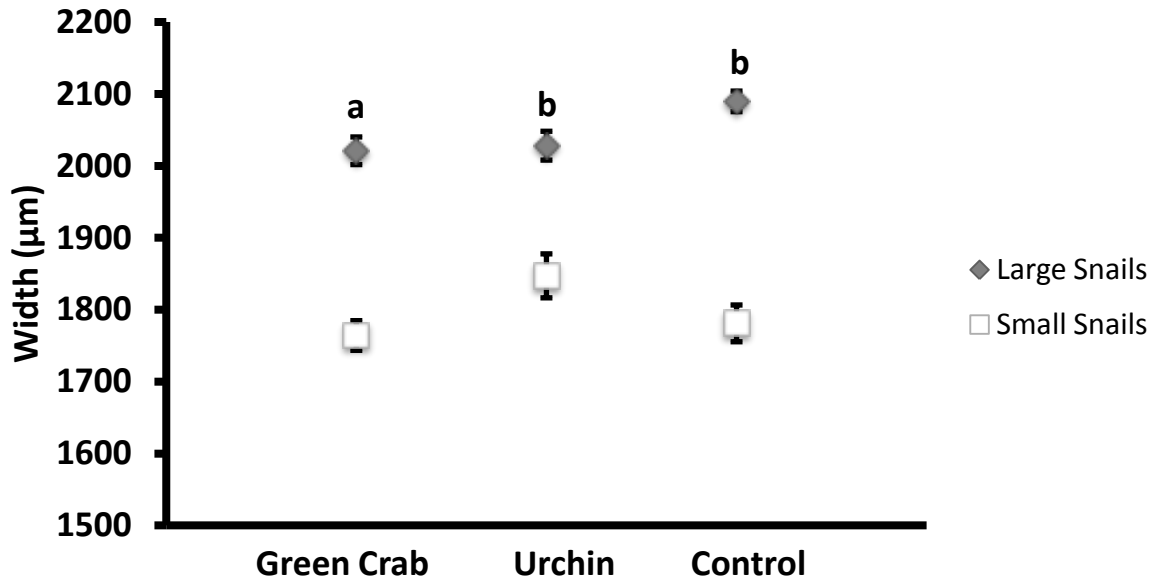


Figure 35. Capsule width by snail size and treatment. large snails laid significantly wider ($\bar{x} = 2046 \mu\text{m}$) egg capsules than small snails ($\bar{x} = 1797 \mu\text{m}$). In the presence of green crabs, snails laid shorter capsules ($\bar{x} = 1892 \mu\text{m}$) than in urchin ($\bar{x} = 1937 \mu\text{m}$) and control ($\bar{x} = 1935 \mu\text{m}$) treatments. Additionally, there appears to be an interaction between snail size and treatment, which is likely the result of small snails in the urchin treatment increasing capsule width, while large snails exposed to this same treatment decreased capsule width in comparison with the control. Error bars represent the standard error; letters indicate statistically significant groups.

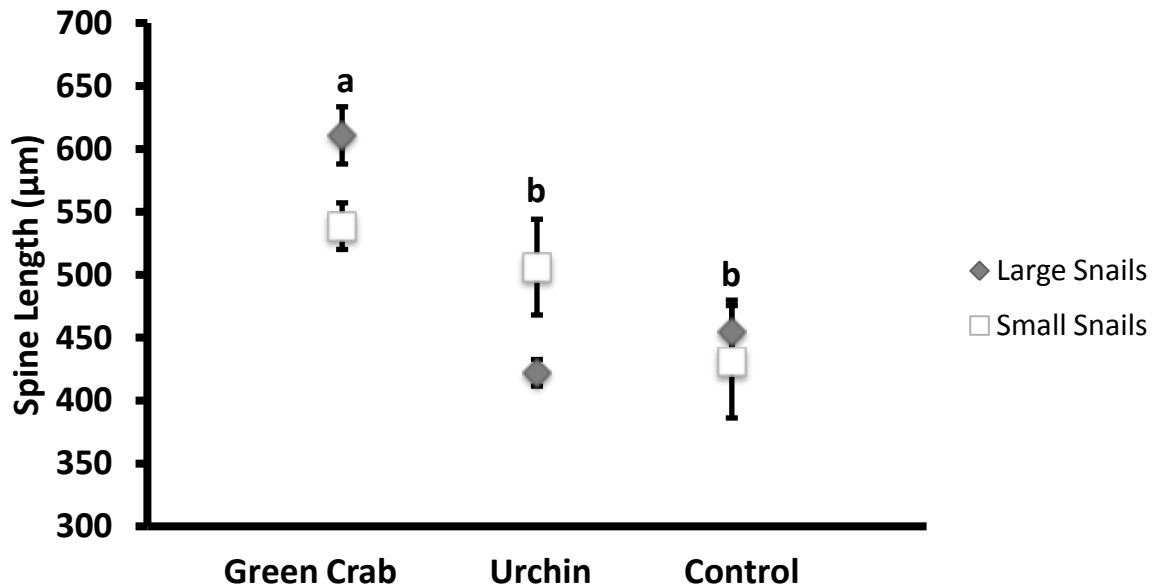


Figure 36. Capsule spine length by snail size and treatment. Spine length did not vary with snail size. However, green crab cue caused snails to increase spine length ($\bar{x} = 575 \mu\text{m}$) in comparison to control snails ($\bar{x} = 443 \mu\text{m}$). Additionally, there was a significant interaction between snail size and treatment, due to small snails in the urchin treatment increasing spine length ($\bar{x} = 506 \mu\text{m}$) significantly in comparison with large snails ($\bar{x} = 422 \mu\text{m}$) in the same treatment. Error bars represent the standard error; letters indicate statistically significant groups.

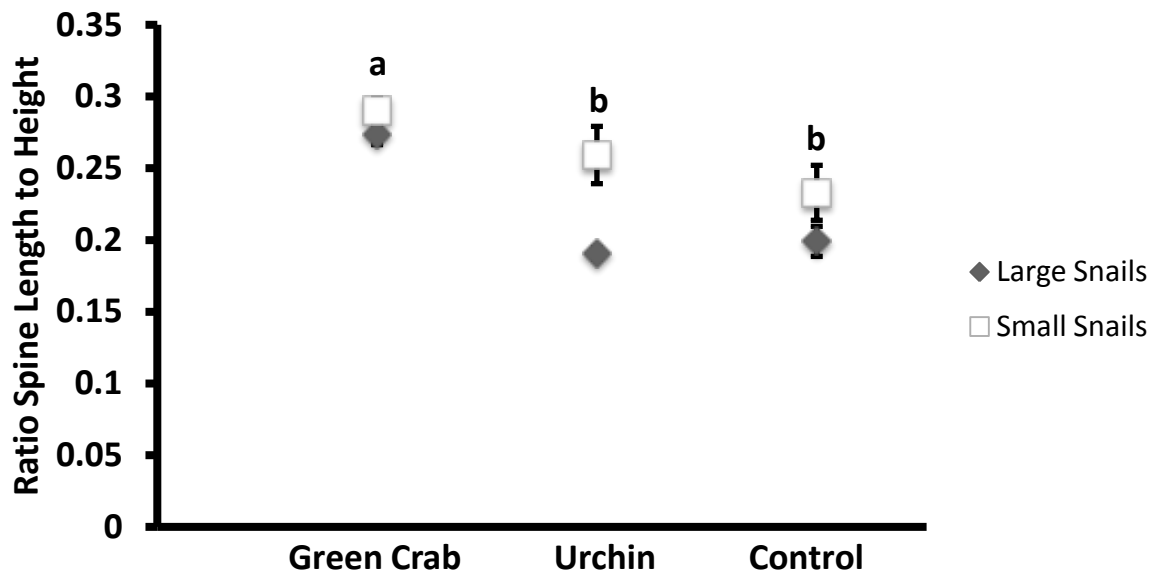


Figure 37. Ratio of spine length to height by snail size and treatment group. Small snails had a higher ratio of spine length to height ($\bar{x} = 0.261$) than large snails ($\bar{x} = 0.221$), suggesting that small snail spines are larger relative to the size of their capsules. Additionally, snails exposed to green crabs had larger spines relative to capsule height ($\bar{x} = 0.282$) than urchin ($\bar{x} = 0.225$) and control ($\bar{x} = 0.216$) treatments. Error bars represent the standard error; letters indicate statistically significant groups.

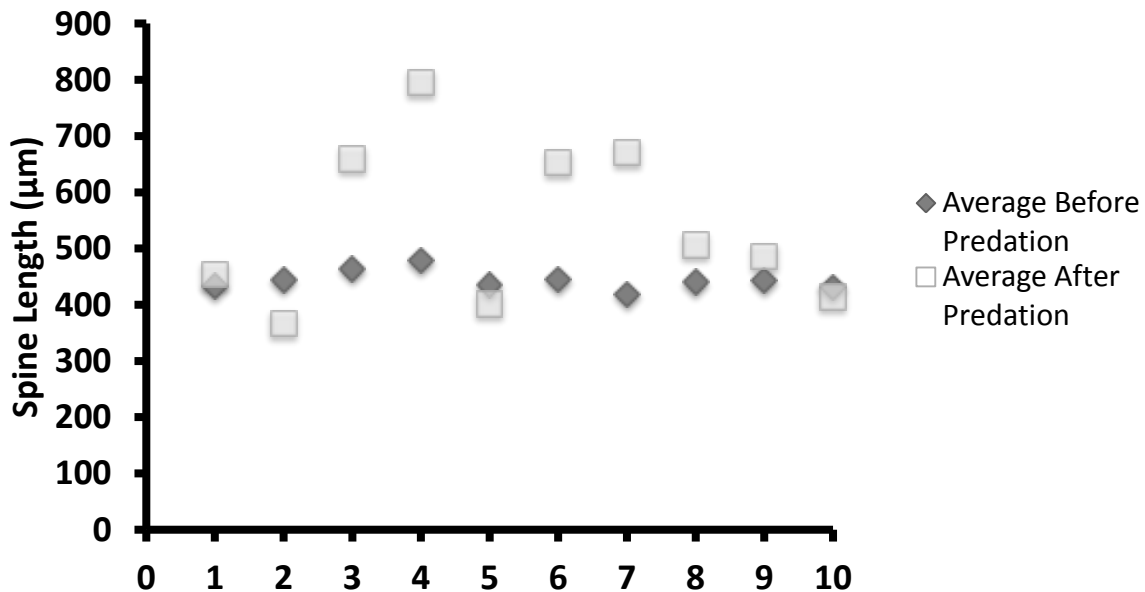


Figure 38. Average spine lengths before and after a predation trial. The average spine length between large- and small-spined capsules before inserting a hermit crab predator was approximately 450 μm . Overall, average spine length increased over the duration of 7 out of 10 trials. These data suggest that hermit crabs preferentially prey upon short-spined egg capsules.

TABLES

Table 1. Comparative results across all three studies.

Comparative Results			
Measure	2010- <i>C. maenas</i> Absent	2010- <i>C. Maenas</i> Present	2011
Egg Capsules	Large and medium size snails laid more than small snails.	Large snails laid more than medium snails.	Significant interaction between size and predator presence.
Eggs Per Capsule	Large and medium size snails laid more than small snails.	No significant differences.	Large snails laid more than small snails. Additional significant interaction between size and predator presence.
Reproductive Output	Large and medium size snails produced more eggs in total during the reproductive season than small snails.	In the presence of green crabs, snails decreased the total number of eggs produced in the reproductive season.	Large size snails produced more eggs in total during the reproductive season than small snails.
Capsule Height	Large and medium size snails laid taller capsules than small snails.	No significant differences.	Large snails laid taller capsules than small snails. Additional significant interaction between size and predator presence.
Capsule Width	Large and medium size snails laid wider capsules than small snails.	Large snails laid wider capsules than medium snails.	Large snails laid wider capsules than small snails. Snails exposed to green crabs decreased capsule width. Additional significant interaction between size and predator presence.
Capsule Spine Length	No significant differences.	No significant differences.	In the presence of green crabs, snails laid capsules with taller spines. Additional significant interaction between size and predator presence.

Shared measures included the quantification of egg capsule number, egg number per capsule, total reproductive output, and capsule height, width, spine length, and the ratio of spine length to height. Note that only significant differences are mentioned here.

Table 2. Two-way and nested two-way ANOVA table of egg capsule number, eggs per capsule, and total reproductive output for 2010

Variable	Factor	df	F	p
Egg Capsule Number	Size	1,11	7.016	0.021
	Treatment	1,11	3.487	0.086
	Size*Treatment	1,11	2.683	0.130
Eggs per Capsule	Size	1,60	0.125	0.725
	Treatment	1,60	3.430	0.069
	Size*Treatment	1,60	3.089	0.084
	Container#(Size (Treatment))	11, 60	0.919	0.528
Total Reproductive Output	Size	1,11	3.764	0.076
	Treatment	1,11	4.977	0.046
	Size*Treatment	1,11	3.750	0.079

Data were analyzed using two-way ANOVAs and nested two-way ANOVAs to determine if there was an effect of size, treatment, or an interaction between the two on each variable. The effect of container number when nested within size when nested within treatment was also analyzed. Significant effects ($p < 0.05$) are listed in bold.

Table 3. Nested two-way ANOVA table of egg capsule height, width, spine length, and the ratio of spine length to height for 2010

Variable	Factor	df	F	p
Capsule Height	Size	1,135	6.723	0.011
	Treatment	1,135	0.305	0.582
	Size*Treatment	1,135	0.590	0.444
	Container#(Size (Treatment))	11,135	1.635	0.096
Capsule Width	Size	1,135	31.533	0.001
	Treatment	1,135	1.774	0.185
	Size*Treatment	1,135	0.477	0.491
	Container#(Size (Treatment))	11,135	1.227	0.275
Spine Length	Size	1,115	0.368	0.545
	Treatment	1,115	1.677	0.198
	Size*Treatment	1,115	0.295	0.588
	Container#(Size (Treatment))	11,115	1.075	0.388
Spine Length to Height	Size	1,115	1.587	0.210
	Treatment	1,115	0.938	0.335
	Size*Treatment	1,115	0.006	0.940
	Container#(Size (Treatment))	11,115	1.085	0.379

Data were analyzed using two-way ANOVAs and nested two-way ANOVAs to determine if there was an effect of size, treatment, or an interaction between the two on each variable. The effect of container number when nested within size when nested within treatment was also analyzed. Significant effects ($p < 0.05$) are listed in bold.

Table 4. Two-way and nested two-way ANOVA table of egg capsule number, eggs per capsule, and total reproductive output for 2011

Variable	Factor	df	F	p
Egg Capsule Number	Size	1, 28	5.326	0.030
	Treatment	2, 27	0.805	0.459
	Size*Treatment	2, 24	5.103	0.014
Eggs Per Capsule	Size	1,270	70.201	0.001
	Treatment	2,270	2.233	0.109
	Size*Treatment	2,270	7.309	0.001
	Container#(Size (Treatment))	24,270	4.972	0.001
Total Reproductive Output	Size	1, 28	15.206	0.001
	Treatment	2, 27	0.574	0.570
	Size*Treatment	2, 24	3.121	0.062

Data were analyzed using two-way ANOVAs and nested two-way ANOVAs to determine if there was an effect of size, treatment, or an interaction between the two on each variable. The effect of container number when nested within size when nested within treatment was also analyzed. Significant effects ($p < 0.05$) are listed in bold.

Table 5. Nested two-way ANOVA table of spine length and the ratio of spine length to height for 2011

Variable	Factor	df	F	p
Spine Length	Size	1,860	0.094	0.759
	Treatment	2,860	41.314	0.001
	Size*Treatment	2,860	13.314	0.001
	Container#(Size (Treatment))	24,860	3.575	0.001
Spine Length to Height	Size	1,860	40.546	0.001
	Treatment	2,860	47.296	0.001
	Size*Treatment	2,860	6.892	0.001
	Container#(Size (Treatment))	24,860	3.193	0.001

Data were analyzed using nested two-way ANOVAs to determine if there was an effect of size, treatment, or an interaction between the two on each variable. The effect of container number when nested within size when nested within treatment was also analyzed. Significant effects ($p < 0.05$) are listed in bold.

Table 6. Logistic regression data table

Variable	β	Sig.	Exp(β)
Hermit Crab #	-0.012	0.828	0.988
Position	-0.007	0.800	0.993
Height	-0.001	0.217	0.999
Width	0.000	0.791	1.000
Spine Length	-0.002	0.011	0.998
β_0	3.822	0.027	45.718

β , significance (p) and Exp(β) values for each variable tested are displayed. These data suggest that hermit crab foraging preferences can be significantly predicted by capsule spine length, while the position, height, and width of a capsule does not appear to influence this preference. Significant effects ($p < 0.05$) are listed in bold.

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